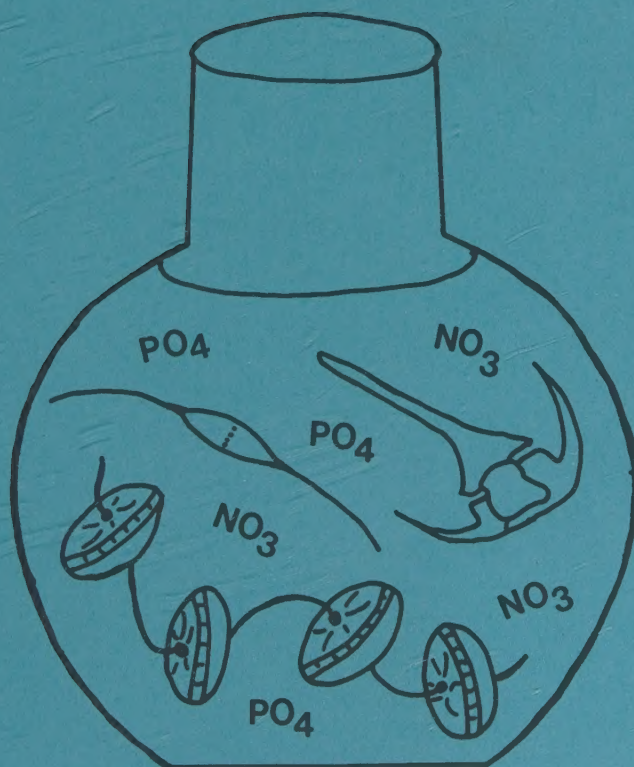




AVD FÖR MARIN BOTANIK LUNDS UNIVERSITET

EXPERIMENTAL INVESTIGATIONS OF LIMITING NUTRIENTS FOR PHYTOPLANKTON PRODUCTION IN THE BRACKISH-WATER ÖRESUND, SW SWEDEN.

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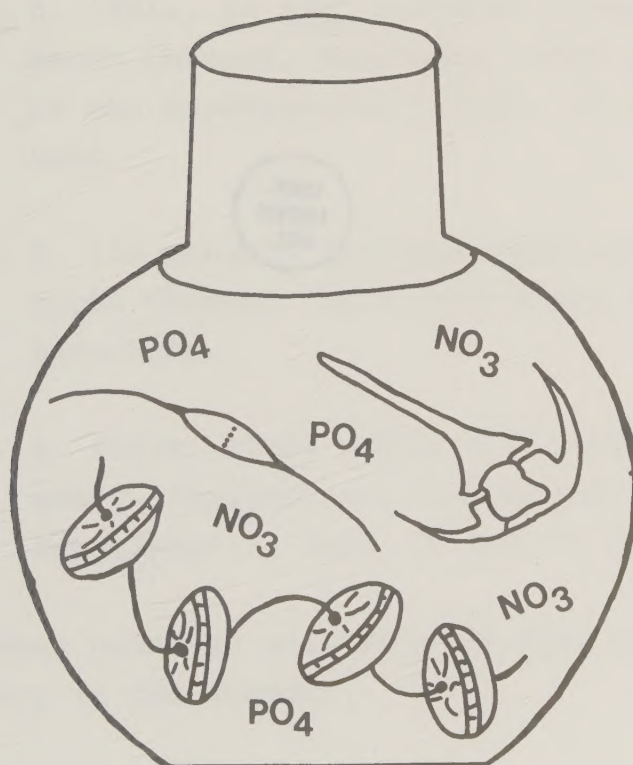
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PLANKTON PRODUCTION IN THE BRACKISH-WATER ÖRESUND, SW SWEDEN

Edna Granéli

The dissertation is a summary of the following published and unpublished papers:

- I Granéli, E. 1978. Algal assay of limiting nutrients for phytoplankton production in the Öresund. - *Vatten* 34:117-128.
- II Granéli, E. 1980. Influence of salinity, phosphorus and nitrogen on the growth of phytoplankton in the Öresund. - Dept. of Marine Botany, Lund.
- III Granéli, E. 1981a. In situ nutrient bioassay in the Falsterbo Channel. Nutrients added at the beginning of the experiments. - Dept. of Marine Botany, Lund.
- IV Granéli, E. (in press). Bioassay experiments in the Falsterbo Channel. Nutrients added daily. - *Meeresforschung*.
- V Granéli, E. 1981b. Algal assay with sewage and Öresund water. Implications for sewage treatment optimization. - Dept. of Marine Botany, Lund.

The above Roman numerals will be used when reference is made to papers in the summary.

INTRODUCTION

Production and biomass of phytoplankton are controlled by several abiotic and biotic factors. Among the former can be mentioned light, temperature, turbulence (sinking rate) and inorganic plant nutrients (e.g. carbon, nitrogen, phosphorus and silica). Important biotic factors are grazing and parasitism. Control mechanisms interact in complicated ways with each other, e.g. light climate is influenced by selfshading and nutrient availability by vertical stratification and grazing.

In the sea nutrient availability is of decisive importance for not only primary, but also secondary and tertiary (e.g. fish) production. Aquatic ecosystems associated with a high net production of biomass seem to be dependent on a constant input of nutrients. Nutrient input occurs in marine environments through upwelling or transport of nutrient-rich water from terrestrial or freshwater ecosystems.

Areas of naturally high nutrient availability are limited, and a large part of the oceans can be regarded as "desert" with extremely low ambient concentrations of ammonia, nitrate and phosphate. The most important commercial fisheries of the world are situated in areas of upwelling. Upwelling is a natural eutrophication. The corresponding cultural eutrophication, i.e. transport of nutrients from land to water through sewage, agricultural drainage and erosion, is often considered a serious threat to aquatic environments (although not un-animously, cf. Steemann Nielsen 1976, Dyrssen 1978).

According to classical theories, nutrients are thought to be consumed and regenerated in the sea more or less in the same proportions as they are found in phytoplankton. In semi-enclosed basins, e.g. the Baltic, this is not the

case, however (Sen Gupta and Koroleff 1973), and phosphate is found in excess over inorganic nitrogen. On the other hand, many unpolluted lakes seem to have a relative deficiency of phosphorus. Sewage and agricultural drainage water generally do not contain nitrogen and phosphorus in proportions optimal for phytoplankton growth. Ratios between these nutrients in strongly polluted recipients may be different from those found in the unpolluted stage.

Unbalanced supply of inorganic nutrients to a water body or slow rate of addition may lead to nutrient-limited growth of phytoplankton, providing other factors, such as light or sinking rate, are not more limiting. According to the principle of Liebig the nutrient in short supply will put an upper limit on biomass accumulation or "formation". For planktonic organisms the situation is more complicated, however, because of constant losses due to sinking, grazing etc. Uptake rates for nutrients and growth rate (at steady state) of planktonic algae have been shown to be hyperbolic functions of ambient nutrient concentrations (e.g. Michaelis-Menten kinetics). The distinction between these two kinds of nutrient limitation is extremely important and has led to different experimental approaches, batch enrichment experiments in the former case and chemostat experiments in the latter. Both types of nutrient limitation are discussed in the dissertation, although batch experiments have been used.

The main objective of the present work was to identify the primary limiting nutrient (*sensu* Liebig) for phytoplankton production in the Öresund and to determine seasonal and spatial (surface VS deeper layers and northern VS southern Öresund) differences with respect to

nutrient limitation. Different kinds of nutrient enrichment algal assays were compared, viz. laboratory tests in bottles with both unialgal cultures and natural phytoplankton populations and bag experiments *in situ*. Several measures of algal response to enrichment were compared, i.e. chlorophyll *a*, plasma volumes, POC, cell numbers, ^{14}C fixation and assimilation numbers. Algae were identified to species to determine what part of the phytoplankton community reacted most strongly to enrichment.

As a consequence of increasing problems with cultural eutrophication, the need to decrease transport to water bodies of nutrients in municipal sewage has been realized. In Sweden over 80% of the urban population is connected to sewage treatment plants with phosphorus removal. Because of differences with respect to nutrient limitation between fresh and salt waters, one would expect sewage treatment in municipalities with a marine outfall to differ from that in municipalities with a freshwater recipient. In Sweden this is not the case, however, and phosphorus removal has been adopted for treatment plants discharging effluents to the Baltic, the Öresund and the Kattegatt. The debate concerning tertiary treatment in the Öresund region inspired this dissertation. In the final article (V) effects of different kinds of sewage on the phytoplankton of the Öresund are discussed in detail.

STUDY AREA

Water for laboratory experiments (I, II, V) was collected from the Öresund proper. The Öresund is a narrow and relatively shallow (max depth 53 m) strait located between the SW coast of Sweden and Zealand in Denmark. It is one of the connections between the Baltic and the Kattegatt.

Water flow through the Öresund is large, net movement being about $120 \times 10^9 \text{ m}^3$ per year to the north. Because of a deeper counter-current with more-saline Kattegat water, which is eventually mixed into the upper layers, total northgoing water transport is on the order of $500 \times 10^9 \text{ m}^3$ per year. Thus water renewal in the Öresund is rapid; mean retention time in the 0-20 m layer is 2-3 days, while deeper layers are renewed more slowly.

Strong salinity gradients, both horizontal and vertical, give the Öresund character of an estuary. The total range of salinities is 8-30 o/oo. Water derived from the Baltic has a rather constant, low salinity (8-10 o/oo). Inflow of deep Kattegat water can increase salinity in the bottom layer of the Öresund to about 30 o/oo.

The Öresund receives sewage from a human population of more than two million including the cities of Copenhagen, Malmö, Helsingborg, Helsingör and Landskrona. The discharge of industrial sewage is considerable, notably from fertilizer industries in the northern part of the Swedish Öresund (Boliden AB, Helsingborg).

The *in situ* experiments (III, IV, V) were performed at the south entrance to the Falsterbo Channel, which is situated at the transition between the Öresund and the Baltic. Water in the channel is not markedly influenced by sewage discharge and is representative for the south Baltic.

EXPERIMENTAL METHODS

Enrichment experiments were made both in bottles in the laboratory using either *Phaeodactylum tricornutum* Bohlin (I, V) or natural phytoplankton populations (I, II, V) as test organisms and *in situ* using natural plankton communities (III, IV, V).

Laboratory experiments were made in 500 ml spherical glass bottles filled with 400 ml medium. Water was filtered through Whatman GF/C glass fiber filters before inoculation with *P. tricornutum*. Filtration through 100-180 μ m nylon nets was used to remove larger zooplankters when tests were made with indigenous phytoplankton populations. Nutrient solutions were prepared from reagent grade chemicals and added singly (phosphate, nitrate, EDTA, trace elements) or in different combinations to the bottles. Final concentrations added corresponded to different dilutions of the Z8 medium, 10% in (I) and (V) and 1% in (II). Added amounts were large in comparison to background nutrient concentrations (added P and N in (II) was 55 and 850 $\text{mg} \cdot \text{m}^{-3}$). Additions of raw or tertiary sewage from the Sjölanda treatment plant in Malmö were made to 1, 5 and 10% concentrations (V). Influence of higher salinity Öresund bottom water on phytoplankton growth in less saline surface water was tested together with additions of N and P (II). Bottles were incubated 4 to 10 days at constant temperature (20°C) and illumination ($0.4 \text{ W} \cdot \text{m}^{-2}$) and were shaken once a day.

In situ experiments were made in 200 l double, transparent polyethylene bags containing 150 l unfiltered surface water from the experimental site, the Falsterbo Channel. The bags were nailed to a float, covered by a transparent roof and anchored at 4 m water depth (III). Phosphate, ammonia and nitrate, alone or in combination, were added daily (IV) or at the start of the experiment (III). Primary, secondary or tertiary sewage from the treatment plants in Lund and Genarp, SW Sweden were added to 1 or 5% concentration (V). Bags were sampled daily for up to two weeks, after which they started to disintegrate.

Standard (manual) methods were used for analysis of nutrient fractions, chlorophyll *a*, ^{14}C fixation (GM counter) etc. Analyses were as far as possible made immediately after sampling without fixatives. POC was analyzed with

an infrared CO₂ analyzer after wet oxidation. An inverted microscope was used for phytoplankton counting, while species identification was made with a phase-contrast microscope. *P. tricornutum* cell numbers were determined with an electronic particle counter.

MEASURES OF BIOMASS RESPONSE

Chlorophyll *a* was used as a measure of phytoplankton biomass throughout the study. As the amount of chlorophyll per unit phytoplankton biomass can be quite variable, comparison was made with other biomass parameters. For *P. tricornutum* cell numbers and chlorophyll *a* concentrations were linearly correlated (I,V). In bag experiment IV phytoplankton plasma volume was calculated after enumeration of the most frequent species. Plasma volume and chlorophyll *a* were not linearly correlated in this experiment over the total range of measured values. For high chlorophyll *a* concentrations ($>10 \text{ mg} \cdot \text{m}^{-3}$) found after enrichment with both nitrogen and phosphorus, plasma volumes did not increase in proportion to chlorophyll *a*. Assuming a conversion factor from plasma volume to cell carbon of 0.11, the C/chl *a* quotient was around 70 for low chlorophyll *a* concentrations and around 30 for high. The same general trend was found in another bag experiment (III) in which total particulate organic carbon (POC) was measured. At the start of this experiment POC/chl *a* quotients were as high as 160-180, but quotients decreased to 50-70 when peak chlorophyll *a* concentrations were reached. In a laboratory experiment good linear correlation between POC and chlorophyll *a* (POC/chl *a* ca 25) was found for both natural populations of Öresund plankton and *P. tricornutum* monocultures, however. Although relations between cell carbon or POC and chlorophyll *a* were variable, these variations were of less magnitude than changes in chlorophyll *a*.

Qualitatively, chlorophyll *a* was therefore a good indication of the biomass response of phytoplankton to enrichment.

MEASURES OF GROWTH RESPONSE

It has been observed in short-term enrichment experiments that ^{14}C fixation does not react immediately to nutrient additions. This phenomenon was also noted here (I). Primary production and yield in terms of chlorophyll *a* were correlated only when ^{14}C fixation was measured after 3-4 days (I, II). Assimilation numbers ($\text{mg C}_{\text{fixed}} \cdot \text{mg chl } a^{-1} \cdot \text{h}^{-1}$) were affected by nutrient and sewage additions, although photosynthetic capacity did not increase as consistently as chlorophyll *a* after enrichment.

RESPONSE AT THE SPECIES LEVEL

Enrichment of Öresund water resulted in rapid successions in the phytoplankton community. This phenomenon was clearly seen in laboratory experiments and occurred even in control bottles. Several phytoplankton species disappeared after one week of incubation (II). Diatoms belonging to the genera *Skeletonema*, *Nitzschia* and *Rhizosolenia* were among those remaining. *S. costatum* often dominated after enrichment (I). This species is common all over the world and forms blooms in nutrient-rich water. In phosphorus-enriched bottles in August the heterocyst-carrying cyanophycean *Nodularia spumigena* became dominant (I, II).

In bag experiments succession was also noted, although total phytoplankton plasma volume did not change greatly in bags without added nutrients (IV). These experiments were conducted in Baltic water (low salinity) during summer,

and unidentified monads made up a substantial fraction of the biovolume. However, the species which reacted most strongly to nitrogen and phosphorus enrichment were diatoms (*Nitzschia closterium*, *S. costatum*, *Chaetoceros wighamii*), green algae (*Oocystis* sp) and blue-green algae (*N. spumigena*, *Aphanothece* sp, *Aphanocapsa* sp, *Gomphosphaeria* sp).

LIMITING NUTRIENTS

In most cases phytoplankton biomass (chlorophyll *a*) increased after nitrogen addition, while phosphorus enrichment had no effect. Phosphate additions did increase chlorophyll *a* concentrations on a few occasions in late summer. These cases were connected with the appearance of blue-green algae as noted above. Nitrogen would thus be regarded as the most-limiting nutrient for phytoplankton biomass *sensu* Liebig both in the Öresund and in adjacent parts of the Baltic and the Kattegat. Because of low inorganic N/P quotients (annual mean around 3 on a weight basis) this is to be expected. With respect to nutrient limitation, the Öresund resembles many other coastal and oceanic areas where there is a shortage of nitrogen.

Nitrogen additions could increase chlorophyll *a* concentrations in Öresund water to about three times the values in control bottles (II, cf. Fig 1). With these excessive nitrogen additions, phosphorus limitation appeared. Very high chlorophyll *a* concentrations could be achieved by simultaneous addition of both nitrogen and phosphorus (I, II, III). This indicates that other elements (e.g. silica and iron) are not normally limiting for phytoplankton production in the Öresund. Laboratory experiments showed that addition of a trace element mixture or EDTA or both to Öresund water did not result in better growth than in control (or phosphorus) bottles (I). Trace elements

or, to a greater degree, EDTA further increased growth only after addition of both nitrogen and phosphorus.

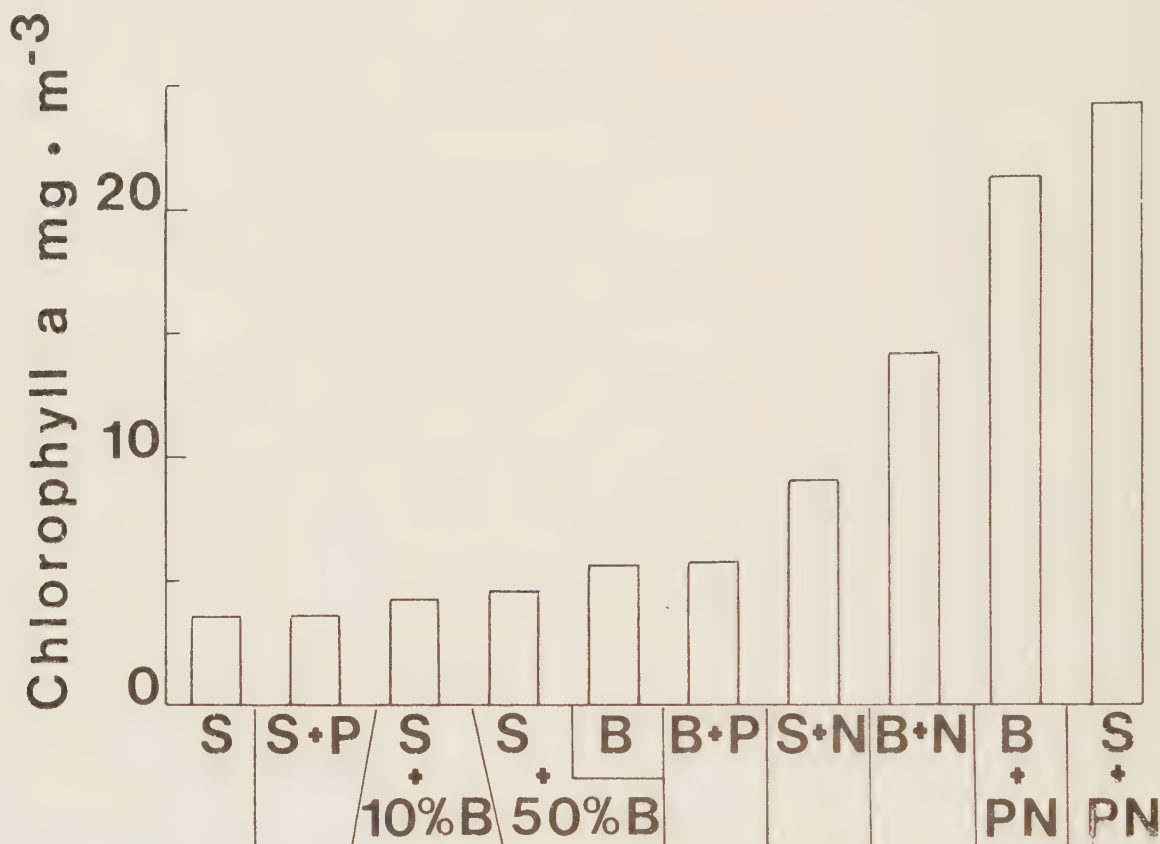


Fig 1. Mean final chlorophyll *a* concentrations in bottle experiments with Öresund surface (S) and bottom (B) water and additions of nitrogen (N) and phosphorus (P). Three stations in the Öresund were sampled on 10 occasions during one year (cf II).

Availability of inorganic nitrogen probably determines potential phytoplankton biomass in the Öresund. However, inorganic nitrogen (and phosphorus) concentrations are seldom extremely low ($<5 \text{ mg} \cdot \text{m}^{-3}$). Measured ammonia and nitrate concentrations were usually higher than half saturation constants for nitrogen uptake and growth of phytoplankton reported in the literature. On the other hand, assimilation numbers were increased on several occasions after additions of nitrogen, nitrogen plus phosphorus or

sewage (III, V), indicating that growth rate was nutrient limited. These experiments were made during the summer when nutrient concentrations in the water were minimal.

Even if phosphorus enrichment seldom increased phytoplankton biomass, phosphorus may stimulate other physiological processes. In bag experiments performed during the summer, additions of phosphate increased N_2 fixation, while inorganic nitrogen seemed to depress this process (Fig 2, unpublished results).

Strong vertical, horizontal and temporal salinity gradients are found in the Öresund. Salinity is therefore an important environmental factor both for macroalgae (von Wachenfeldt 1975) and phytoplankton, and the influence on species composition is evident (Edler 1977). However, the experiments did not verify that salinity also determines the total biomass of phytoplankton (II and Fig 3). Nutrient concentrations are likely to be of greater importance.

NUTRIENT UPTAKE

Added dissolved inorganic nutrients rapidly disappeared from the water (IV, V), even when little growth occurred (as when phosphorus was added). The most rapid nutrient uptake was found during exponential chlorophyll a increase, however. When all added phosphorus and nitrogen had been utilized, phytoplankton populations often collapsed. This happened within 10 days after enrichment and indicates that nutrients were not recycled in the bag experiments. Added phosphate was transformed to particulate organic phosphorus (POP) and dissolved organic phosphorus even without a simultaneous nitrogen addition (III). As a consequence the POP/chl a quotient increased tremendously, from less than 1 to over 10, indicating luxury consumption. Ammonia was taken up in preference to nitrate (V). The

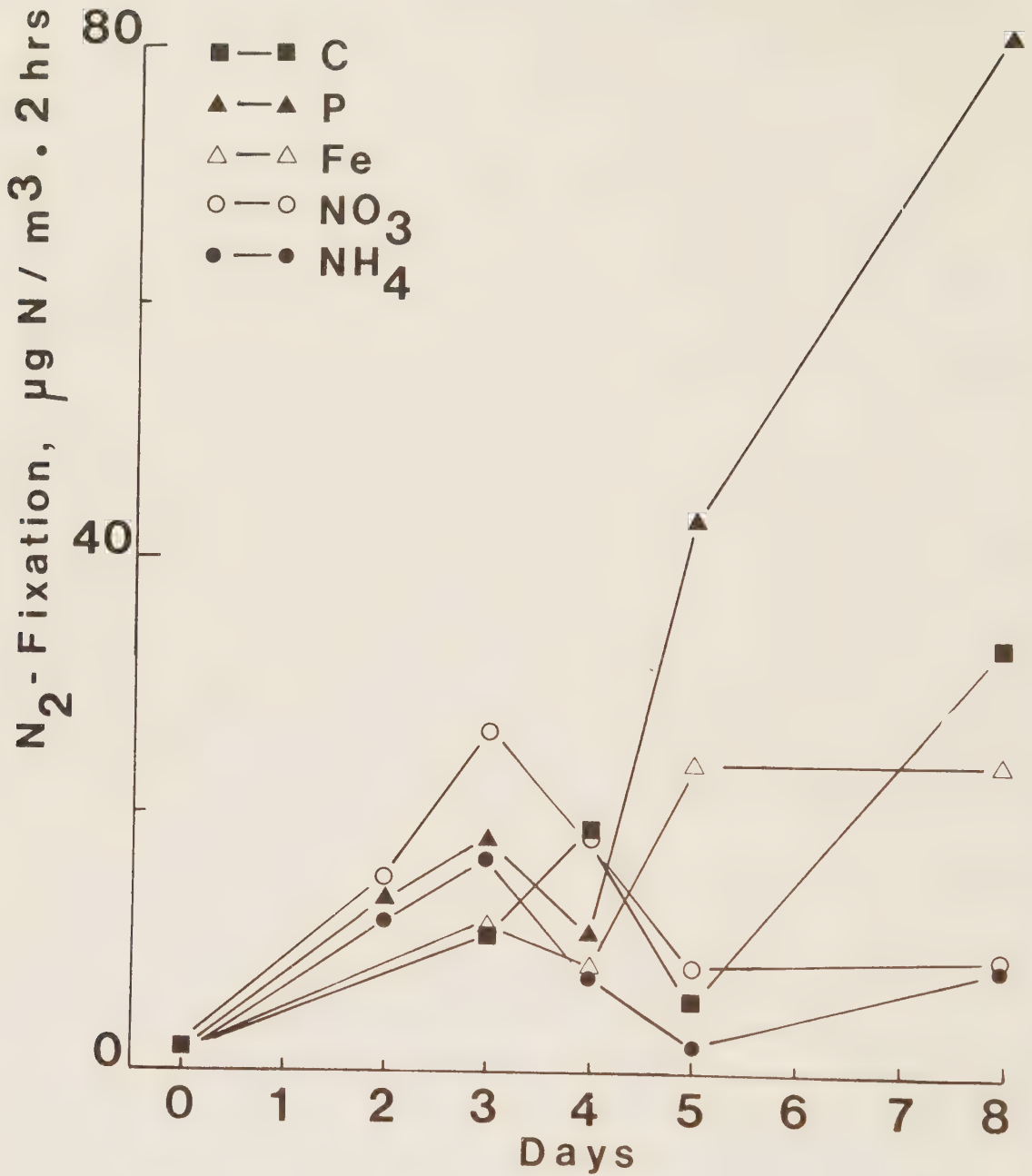


Fig 2. Nitrogen fixation (acetylene reduction method) in bags exposed in the Falsterbo Channel 1978-08-14- -- 1978-08-23. C= control (no additions), P= phosphate addition, Fe= iron addition, NO₃= nitrate addition and NH₄= ammonia addition (Granéli and Sundström unpublished).

ratio between uptake of inorganic nitrogen and uptake of phosphate varied greatly (V). As the experiments were of batch type, no steady state with respect to phytoplankton biomass or ambient nutrient concentrations was achieved.

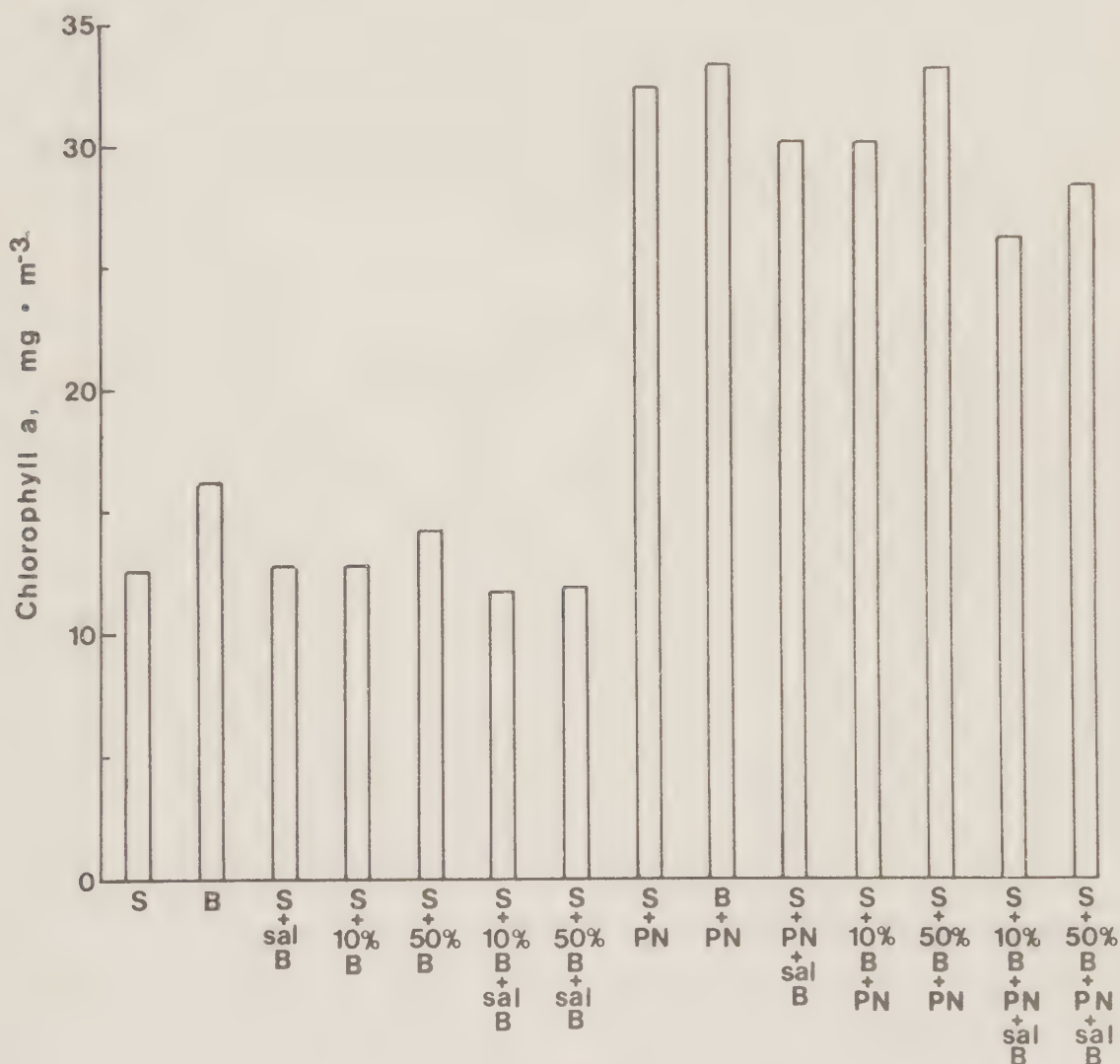


Fig 3. Chlorophyll *a* produced in bottle experiments with different mixtures of Öresund surface water (S) (salinity 17⁰/oo), Öresund bottom water (B) (salinity 30⁰/oo). Additions were salt (NaCl), nitrogen (N) and phosphorus (P). Experiment performed 1979-03-13 -- 1979-03-17. (Granéli unpublished)

EFFECTS OF SEWAGE ON THE RECIPIENT

Sewage additions to Öresund water generally resulted in increased algal growth (V). In laboratory experiments with raw and tertiary sewage from Sjölungda treatment plant (Malmö), phosphorus-depleted tertiary sewage had a lower stimulating effect than corresponding raw sewage. At the lowest (and most realistic) addition tested (1 % sewage), chlorophyll *a* yield decreased by an average of only 30 %, although phosphate in tertiary sewage was one-third of the concentration in raw sewage.

In bag experiments with addition of primary sewage, phosphate was in excess at chlorophyll *a* peaks, while inorganic nitrogen (nitrate) was in excess after tertiary sewage additions. These experiments were also made with high concentrations of sewage (1 and 5 %). Discharge of tertiary sewage to bays in the Öresund with slow water renewal can lead to relative phosphorus deficiency, thus reversing the limiting nutrient situation (Fig 4, Granéli and Sundbäck unpublished). At higher dilutions tertiary sewage discharge would only tend to adjust the low inorganic N/P quotient in the Öresund to values optimal for phytoplankton growth. N/P in Baltic and oceanic phytoplankton is 6 or higher. Thus, it would be difficult to induce phosphate limitation in the central Öresund (where sewage is likely to be diluted a minimum of 500 times), even through phosphorus removal from all municipal and industrial sewage entering the strait. This is because inorganic N/P quotients are low in Baltic water flowing to the Öresund, a situation which is probably natural and not due to pollution (V). Nitrogen removal in sewage would be more advantageous, as potential algal biomass in the Öresund is proportional to nitrogen concentrations (II). However, nitrogen is more difficult to control since a large fraction is derived from diffuse sources, notably agricultural fertilizer used in the catchment area.

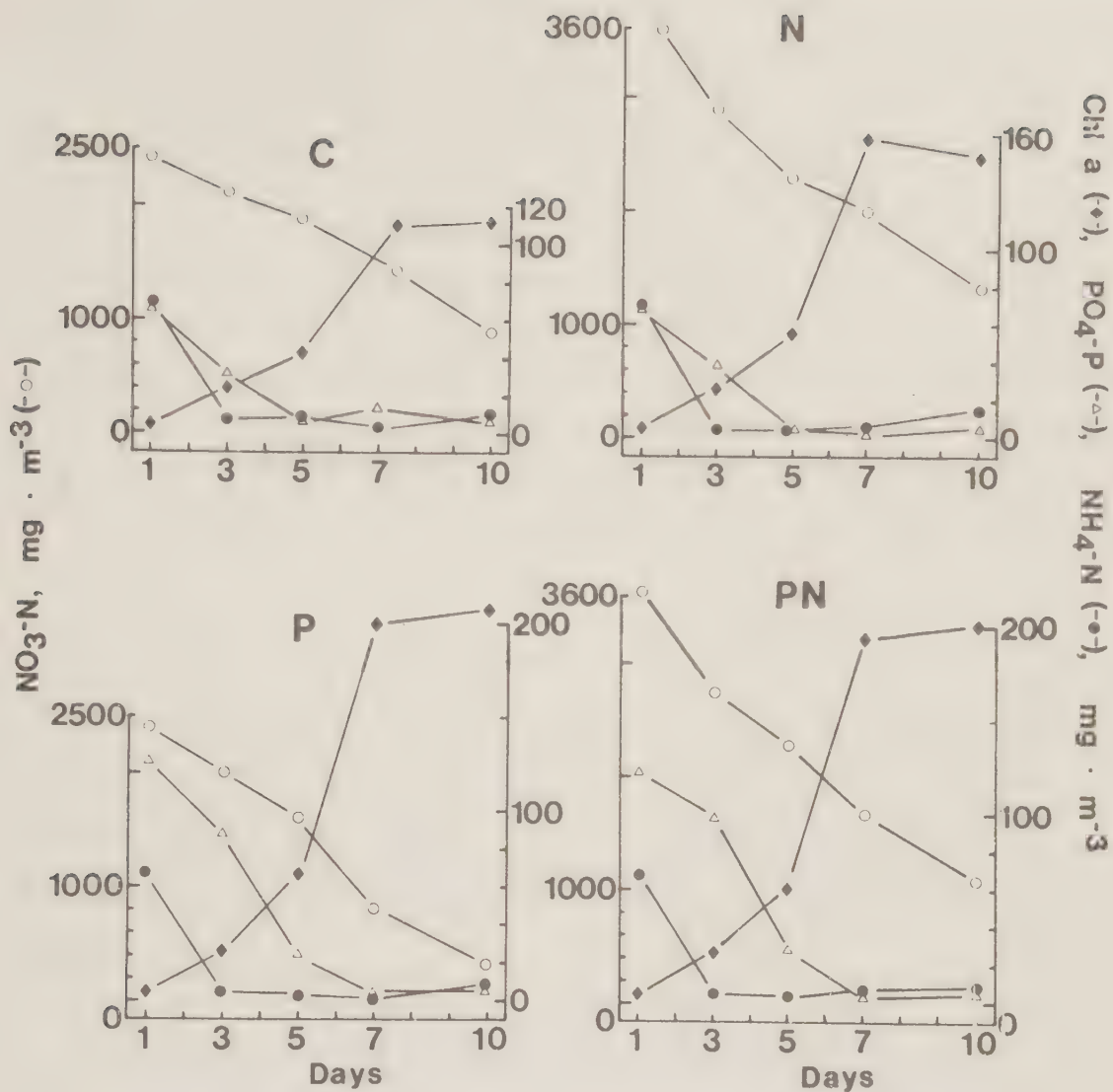


Fig 4. Enrichment experiments in bottles with water from the polluted Lomma bay (recipient for the Sjöblunda tertiary treatment plant) sampled 1979-09-28. C= control (without additions), N= nitrate addition, P= phosphate addition (Granéli and Sundbäck unpublished).

CONCLUSIONS

According to the classical principle of Liebig, nitrogen is the most-limiting nutrient for potential phytoplankton bio-

mass in the Öresund and adjacent waters. It is, however, uncertain if ambient nutrient concentrations are ever low enough to limit phytoplankton growth rates. In *in situ* bag experiments chlorophyll *a* synthesis was stimulated more by enrichment than was phytoplankton biomass. This discrepancy was not noted in laboratory experiments in bottles, however. In enrichment experiments with Öresund water, species selection occurred favouring a few genera of diatoms and also blue-green algae during summer. Present sewage treatment policy on the Swedish side of the Öresund is - with respect to eutrophication control - not optimal. Nitrogen control should be adopted instead of phosphorus removal.

RESUMO EM PORTUGUÊS

Bioensaios de enriquecimento usando a água do Öresund foram realizados com garrafas no laboratório e com sacolas plásticas de 200 l de volume *in situ*. Como organismos testes foram usados as populações naturais fitoplanctônicas e *Phaeodactylum tricornutum*. Adições de nitrogênio estimularam significativamente a biomassa do fitoplancton (clorofila *a*, carbono orgânico particulado, volume plasmático), acima dos valores do controle (sem adições). Este efeito foi observado nas experiências usando água da superfície do Öresund (origem Mar Báltico) e na água de fundo (origem Mar do Kattegat); no norte, na parte central (mais poluída) e no sul (água limpa) do estreito. Adições de fósforo somente aumentaram a biomassa fitoplanctônica e a produção primária ocasionalmente no fim do verão quando algas cianofíceas-heterocísticas se apresentavam em floração (bloom). Números assimilatórios aumentaram consideravelmente todas as vezes em que nitrogênio foi adicionado sozinho ou junto com fósforo. Pode-se concluir então que nitrogênio é o nutriente mais limitante ao crescimento do fitoplancton no Öresund e águas circundantes. Esta conclusão se confirma através dos quocientes entre N/P no Öresund (média= 3).

Experimentos usando água de esgoto como fertilizante e cálculos teóricos, mostram que a remoção de nitrogênio do esgoto municipal desaguando no Öresund, diminuirá o potencial de multiplicação das algas mais do que a remoção de fósforo o fará.

ACKNOWLEDGEMENTS

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**ALGAL ASSAY OF LIMITING NUTRIENTS FOR
PHYTOPLANKTON PRODUCTION IN
THE ÖRESUND**

By Edna Granéli

ALGAL ASSAY OF LIMITING NUTRIENTS FOR PHYTOPLANKTON PRODUCTION IN THE ÖRESUND

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Introduction

The shallow and narrow strait of brackish water connecting the Baltic with Kattegat — the Öresund — suffers heavy sewage input from both municipal and industrial sources. The domestic discharge, mainly from the cities of Copenhagen, Malmö, Landskrona, Helsingborg and Helsingør, corresponds to a population of about 2 million people. Much of this discharge is still through primary treatment plants. On the Swedish side of the Öresund most of the municipal sewage will be tertiary treated (phosphorus removal) in the near future. On the other hand, there is a large industrial discharge of phosphorus from Sweden. Joint Swedo-Danish projects to study the pollution and eutrophication of the Öresund have been running for several years (see e.g. Anonymous 1976).

Eutrophication of the Öresund has been studied through analyses of nutrients, macrobenthic algae and phytoplankton biomass and productivity (von Wachenfeldt 1975, Steemann Nielsen et al. 1976, Edler 1977). To get a more direct indication of the stimulatory effect of nutrient additions to the Öresund, bioassay experiments were performed using both unialgal cultures and natural phytoplankton populations.

Different ways of measuring the response of the algae to the nutrient additions were tested, as algal assays can give different answers depending on the method chosen (Gerhart and Likens 1975).

Materials and methods

Surface samples were taken on ten occasions from April 1976 to April 1977 and poured into 5 l plastic containers. Initially, five different stations were sampled (Fig. 1), but as the results were similar for all stations, samples were taken only at station 1 during most of the investigation period. This station is situated close to Barsebäck, but is not directly influenced by sewage discharge.

The bioassay experiments were started immediately upon return to the laboratory. Water for bioassay with the natural phytoplankton populations was filtered through a 100 µm nylon net to remove large zooplankton, while water for incubation with test algae was filtered through a Whatman GF/C filter (retention efficiency approx. 1 µm). The test alga was *Phaeodactylum tricornutum* Bohlin. The inoculum was taken from a stock culture (10 % Z8 solution) in the exponential growth phase giving an initial concentration of $0.5\text{--}1.5 \times 10^6$ cells $\cdot$ l⁻¹. The bioassay experiments were performed in 500 ml spherical glass flasks filled with 300 ml medium and incubated at 20°C $\pm$ 1 and at $4 \cdot 10^{-2}$ mW $\cdot$ cm⁻² (approx. 4000 lux, 40W Asea-Scandia fluorescent lamps). The flasks were shaken once a day.

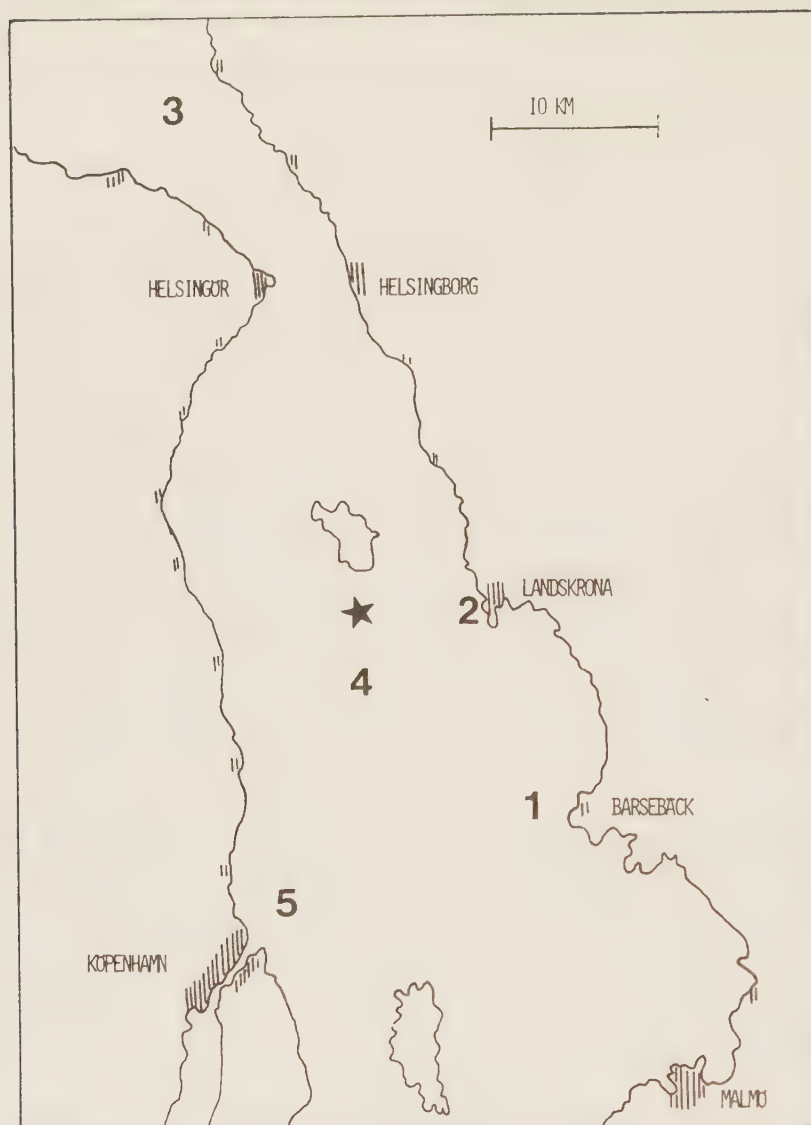


Fig. 1. Sampling stations in the Öresund. ★ = sampling station for phosphorus and nitrogen.

Reagent grade chemicals were used to prepare concentrated stock solutions of nutrients. Small amounts of these solutions, giving final concentrations corresponding to the 10 ‰ Z8 medium (Skulberg 1964, Gargas and Pedersen 1974), were added to the bioassay flasks. Four different solutions were used alone or in combinations: P (final conc. $0.56 \text{ mg PO}_4\text{-P} \cdot \text{l}^{-1}$), N ($8.40 \text{ mg NO}_3\text{-N} \cdot \text{l}^{-1}$), E ($0.37 \text{ mg EDTA} \cdot \text{l}^{-1}$) and T, a trace element mixture (see Gargas and Pedersen 1974).

Once every two weeks measurements of the concentrations of inorganic phosphorus and nitrogen ($\text{NO}_3\text{-N} + \text{NO}_2\text{-N} + \text{NH}_4\text{-N}$) in the Öresund were obtained through the Swedo-Danish Belt project (unpublished results). Samples for these measurements were taken south of the island of Ven (Fig. 1). Inorganic phosphorus and nitrogen in the bioassay flasks were measured at the end of two series (station 1 and 4, April 3, 1976) to check the uptake of the added nutrients.

The response of the algae to the added nutrients was measured as cell number (*Phaeodactylum* only), chlorophyll *a* and ^{14}C uptake. Cell number was determined electronically using a Celloscope model 302 particle counter; chlorophyll *a* was measured ac-

ALGAL ASSAY OF LIMITING NUTRIENTS

according to Lorenzen (1967) and ^{14}C uptake according to Steemann Nielsen (1952). ^{14}C uptake was measured on 30 ml subsamples taken from the bioassay flasks and exposed for two hours to the same light intensity as the flasks. Incubations were routinely done on the fourth day after the beginning of the experiments, but some incubations were also made during the first two days. Radioactivity was measured on a GM-counter. ^{14}C uptake was transformed to $\mu\text{g C} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$ using measurements of pH, temperature and salinity and the formulae in Gargas (1975 a).

The experiments were terminated after 8–10 days and chlorophyll *a* and cell number determined. Dominant phytoplankton species of the natural populations were identified at the beginning and end of the experiments.

Results

Water from stations 1–5 was tested with 12 different nutrient combinations in addition to a control with no additions. The test organism was *Phaeodactylum tricornutum*. As all series gave similar results, only those from two stations are presented (Fig. 2). Growth response in these experiments was measured as chlorophyll *a*.

The control culture (C) and the cultures with additions of phosphorus (P), trace element mixture (T), organic chelator (EDTA, E) or the combinations of phosphorus and trace elements or EDTA (PT and PE) showed similar growth in terms of chlorophyll *a*. Thus addition of these elements alone or in combination to water from the Öresund did not stimulate growth.

Additions of nitrogen (N) or nitrogen in combination with trace elements or EDTA (NT and NE) were weakly stimulative to the growth of *Phaeodactylum*. Heavy growth occurred when both phosphorus and nitrogen (PN) were added. EDTA increased this response somewhat (PNE), while trace elements had no further effect (PNT). Thus the "complete" addition of nutrients (PNTE) had the same effect as phosphorus+nitrogen+chelator.

If nitrogen and phosphorus were not added in combination, there was little uptake of either nutrient, indicating that in the *Phaeodactylum* bioassay both elements were growth limiting.

On the basis of these experiments five different nutrient additions besides the control (C) were chosen for further studies viz. P, N, PN, PNE and PNTE.

Bioassay tests with water from station 1 during one year (April 24, 1976 to April 19, 1977) using *Phaeodactylum* agreed with the above results. There was no stimulation with P alone, a weak stimulation with N alone and strong growth with P and N in combination (Fig. 3). Addition of trace elements and/or EDTA increased the response somewhat.

The natural phytoplankton populations reacted in a similar way, although there were marked seasonal differences in the response (Fig. 4). P alone had no effect in comparison with the control, while N increased growth during spring (March–June). During the rest of the year N had only minor effects. The response was stronger for the other additions (PN, PNE and PNTE) but followed the same trend. However, with PNE and PNTE additions there was also strong growth in the January sample.

The phytoplankton species composition changed in the flasks containing the natural populations. At the end of the experiments one or two species were completely dominant (Table 1). These species were usually not dominant at the beginning of the experiments but were always recorded in plankton samples from the same date and station. Response was similar whether measured as number of cells (for *Phaeodactylum* only),

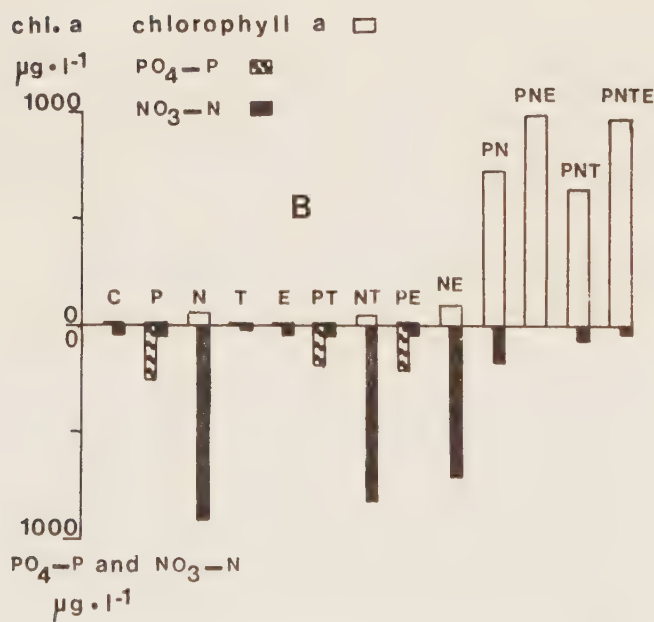
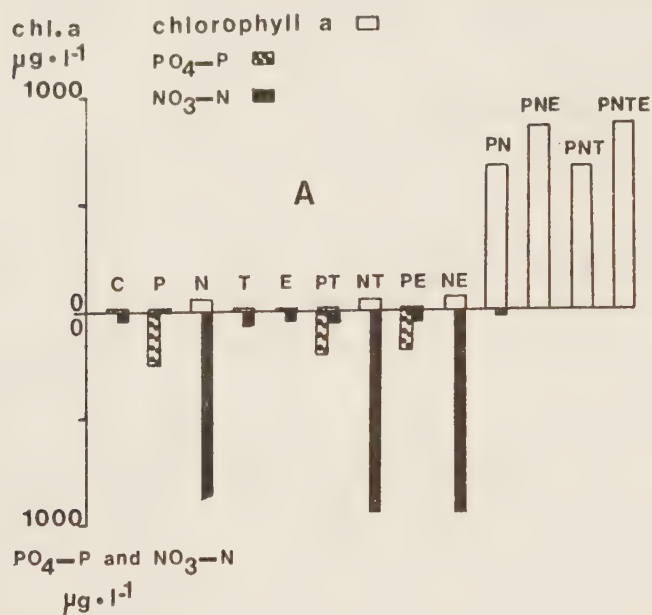


Fig. 2. Bioassay with *Phaeodactylum tricornutum*, April 2, 1976 to April 12, 1976. Response to different nutrient additions measured as chlorophyll *a*. Downward bars: $\text{PO}_4\text{-P}$ and $\text{NO}_3\text{-N}$ remaining in the water at the end of the experiment. C=control, P=phosphorus, N=nitrogen, E=EDTA, and T=trace elements additions. (A) Station 1, (B) Station 4.

chlorophyll *a* or ^{14}C uptake (Fig. 5). Thus there was a good linear correlation between cell number and chlorophyll *a* for *Phaeodactylum tricornutum* (Fig. 6). Chlorophyll *a* (measured at the end of the experiments) and ^{14}C uptake were also correlated for the natural populations, but only if ^{14}C uptake was measured after 4 days of incubation (Fig. 7). When it was measured during the first two days after nutrient addition, there was no correlation with final yield.

Both inorganic phosphorus ($\text{PO}_4\text{-P}$) and inorganic nitrogen ($\text{NO}_3\text{-N} + \text{NO}_2\text{-N} + \text{NH}_4\text{-N}$) showed low concentrations in the Öresund from April to September 1976 (Fig. 8).

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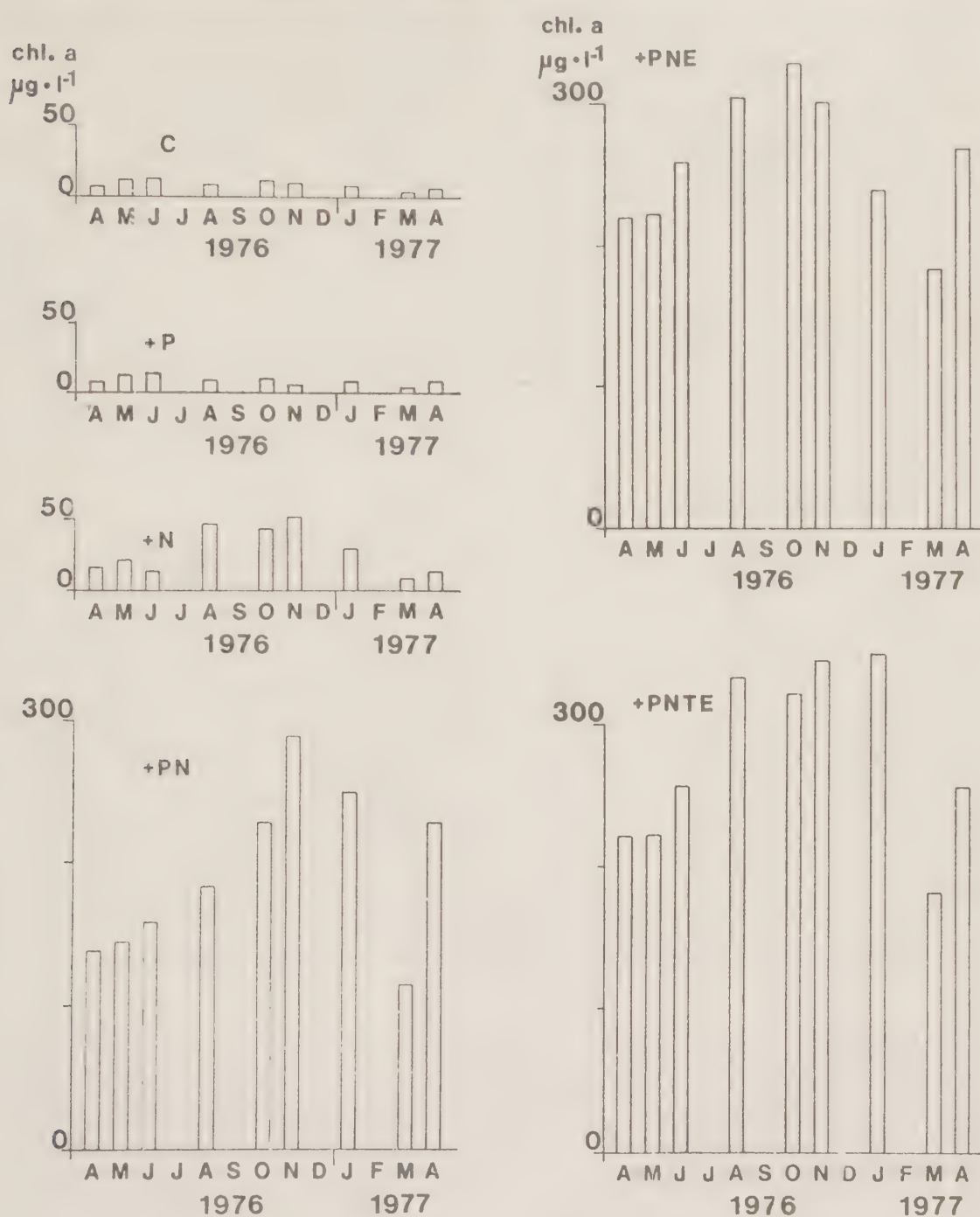


Fig. 3. Seasonal variation in the response of *Phaeodactylum tricornutum* (measured as chlorophyll *a*) to different nutrient additions. Additions as in Fig. 2.

Phosphorus remained low during the rest of the investigated period, while inorganic nitrogen increased during October 1976. After a decline in the inorganic nitrogen concentration in November, there was a sharp increase in the beginning of December with maximum values in the beginning of January (ca $200 \mu\text{g N}\cdot\text{l}^{-1}$). Nitrogen remained high until April 1977.

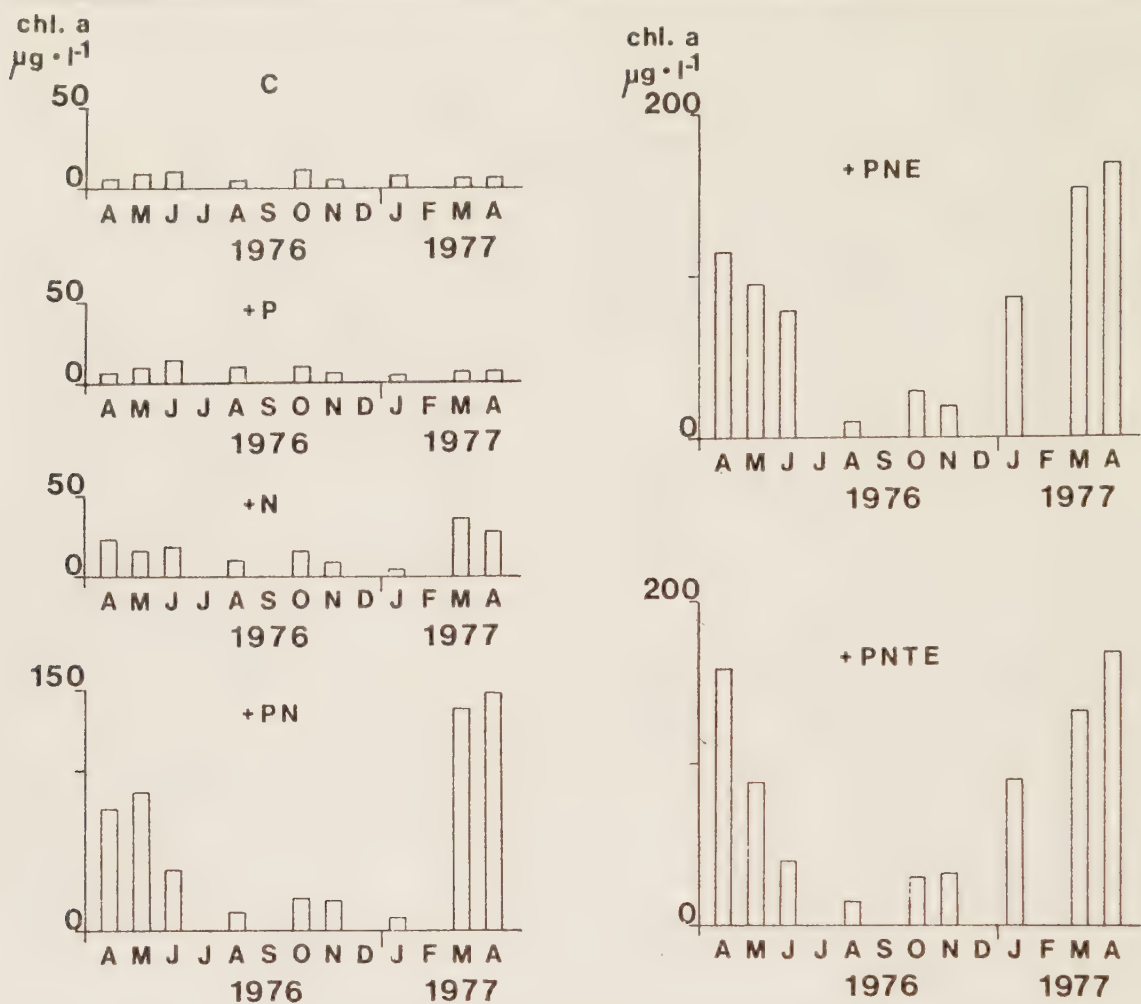


Fig. 4. Seasonal variation in the response of the natural phytoplankton populations to different nutrient additions.

Discussion

Phytoplankton growth in a body of water as a result of nutrient additions has been investigated in several ways. Evidence of the eutrophication process based on measurements in a water body undergoing cultural eutrophication requires several years of

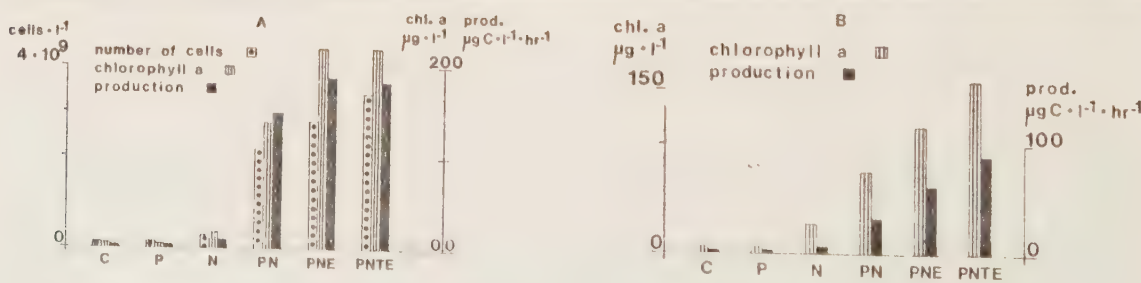


Fig. 5. Bioassay with *Phacodactylum tricornutum* (A) and the natural phytoplankton populations (B) April 24, 1976 to May 03, 1976. Station 1. Response measured as cell number (*Phacodactylum* only), chlorophyll *a* and ¹⁴C uptake.

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Table 1. Dominating phytoplankton species at the start and end of the bioassay experiments with natural phytoplankton populations.

Date	Start	End
April 24, 1976	<i>Skeletonema costatum</i> <i>Dinobryon balticum</i>	<i>Rhizosolenia alata</i> <i>Skeletonema costatum</i>
May 17, 1976	<i>Rhizosolenia alata</i> <i>Skeletonema costatum</i> <i>Chaetoceros constrictus</i> <i>Chaetoceros diadema</i>	<i>Rhizosolenia alata</i> <i>Skeletonema costatum</i>
June 30, 1976	<i>Rhizosolenia alata</i> <i>Chaetoceros danicus</i>	<i>Ceratium tripos</i>
August 26, 1976	<i>Skeletonema costatum</i> <i>Chaetoceros perpusillus</i>	<i>Nodularia spumigena</i>
October 05, 1976	<i>Coscinodiscus granii</i> <i>Chaetoceros radians</i> <i>Skeletonema costatum</i> <i>Cerataulina pelagica</i>	<i>Ceratium furca</i> Monads
November 05, 1976	<i>Nitzschia pseudodelicatissima</i> <i>Skeletonema costatum</i> <i>Rhizosolenia delicatula</i>	Monads
January 26, 1977	<i>Chaetoceros laciniosus</i> <i>Actinocyclus octonarius</i> <i>Skeletonema costatum</i> <i>Nitzschia longissima</i> <i>Ceratium tripos</i>	<i>Nitzschia</i> sp.
Mars 17, 1977	<i>Chaetoceros diadema</i> <i>Chaetoceros similis</i> <i>Chaetoceros laciniosus</i> <i>Rhizosolenia semispina</i> <i>Phaeocystis pouchetii</i>	<i>Skeletonema costatum</i>
April 19, 1977	<i>Detonula confervoides</i> <i>Dinobryon balticum</i>	<i>Nitzschia seriata</i>

observation. Further, there are usually not enough data from the pre-eutrophication period to draw firmly-based conclusions. The least objectionable but usually impractical methods are »whole lake» experiments (Schindler et al. 1971) or experiments with large enclosures suspended in the water (Schelske and Stoermer 1971, Lund 1972, Gerhart and Likens 1975). However, most enrichment studies have been made as bottle tests either in situ or in the laboratory.

Batch algal assays utilizing either special test algae or the natural phytoplankton populations are easily performed in the laboratory (Skulberg 1967, Forsberg 1972 a, b), but have met some objections. Thus nutrient limitation can occur in the bioassay flasks without any correspondence to the situation in the actual water body. Laboratory experiments with nutrient additions may also be of too short duration to give any information on the final effect of such additions to the water body. This is especially true with respect to phytoplankton species composition after a long period of fertilization.

Gerhart and Likens (1975) concluded that different kinds of enrichment experiments yield different results. Especially the short-term ^{14}C bioassay showed to be unsensitive to e.g. phosphorus enrichment. Similar results were reached by Hamilton (1969) and

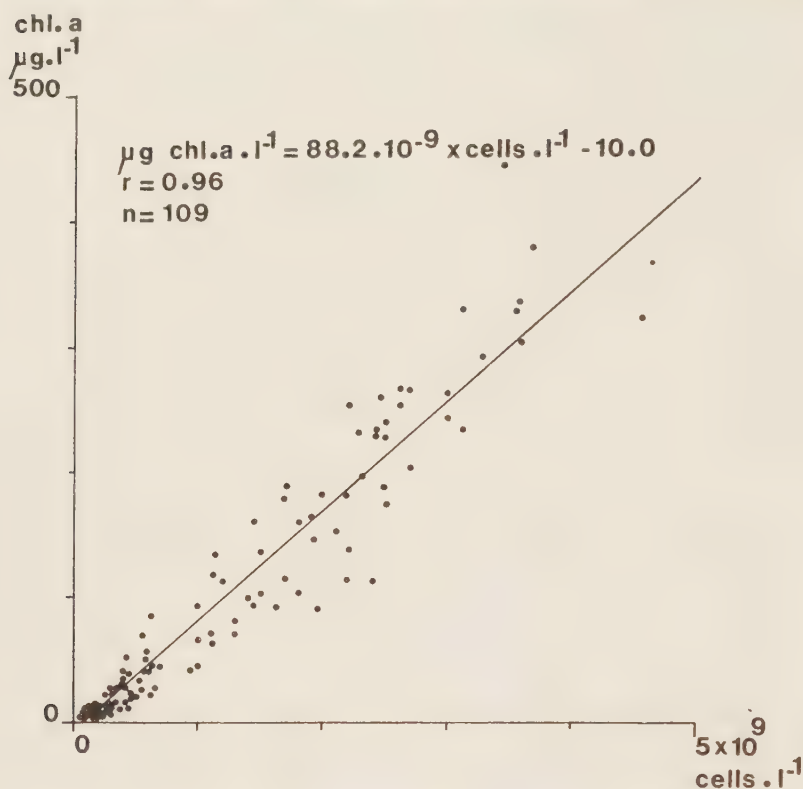


Fig. 6. Correlation between cell number and chlorophyll a for *Phaeodactylum tricornutum*.

O'Brien and Denoyelles (1976). Carlucci and Silbernagel (1969) reported a lag phase between the addition of vitamins to marine phytoplankton and the response measured by ¹⁴C uptake. My experiments support these findings. No increase in ¹⁴C uptake over the control was found in short-term experiments made during the first two days after the addition of nutrients (Fig. 7). The mechanism behind this delayed stimula-

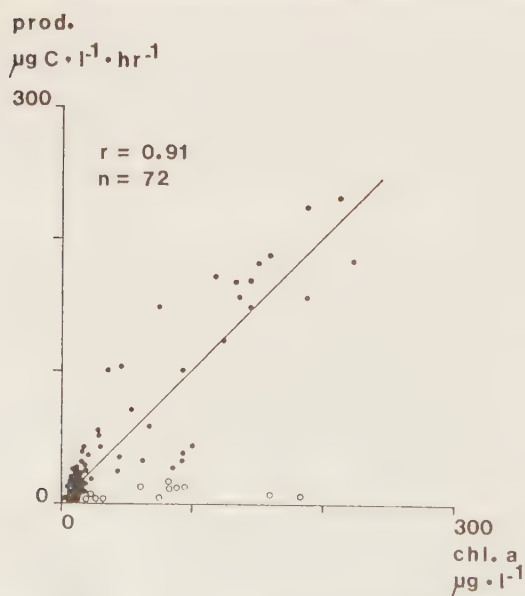


Fig. 7. Correlation between chlorophyll a and ¹⁴C uptake for the natural phytoplankton populations. Open circles: measurements of ¹⁴C uptake made during the first two days after the start of the experiments. Filled circles: measurements after 4 days. Chlorophyll a measured after 8–10 days.

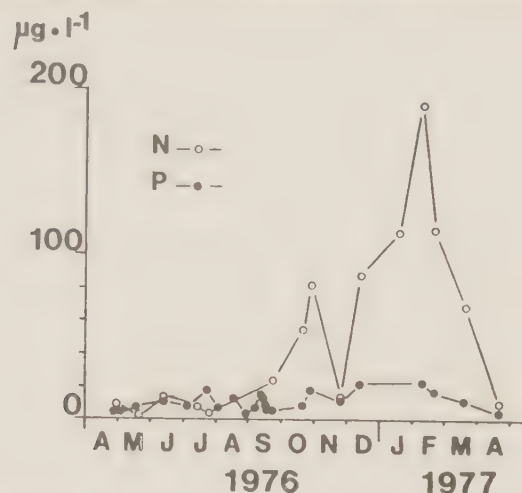


Fig. 8. Seasonal variation in inorganic phosphorus and nitrogen in the surface water of the Öresund. Sampling station according to Fig. 1.

tion or even suppression of carbon uptake is not yet known. Therefore, it seems advisable not to use short-term ^{14}C bioassay in enrichment tests. However, if the algae are left for several days to adapt to the added nutrients, ^{14}C uptake can be used instead of e.g. chlorophyll analysis. The latter is preferred, however as it is simple and faster.

When using a unicellular algae as test organism several growth measurements are possible. Common methods are turbidity measurements, COD or POC — analysis, measurement of dry weight, cell number or chlorophyll. Determination of cell number is rapid if a particle counter is available. In the present investigation cell number and chlorophyll *a* were used for the *Phaeodactylum* tests, and there was a good correlation between these two parameters (Fig. 6). However, under other experimental conditions (different light, temperature, etc.) the relationship might not have been the same.

The enrichment tests, both with *Phaeodactylum* and the natural phytoplankton populations, indicated that phosphorus as well as nitrogen limits phytoplankton growth in the Öresund. However, nitrogen additions alone had a stimulatory effect (Fig. 2 and 3), and from the concentrations of inorganic phosphorus and nitrogen in the surface water of the Öresund (Fig. 8) it can be concluded that nitrogen is likely to limit phytoplankton growth. The weight ratio of inorganic N:P in the Öresund varied during the investigated period between 0.1:1 and 8.2:1 with a mean of 3.5:1, i.e. under the ratio of about 4.5:1 suggested by Ryther and Dunstan (1971) as a reasonable mean value for marine phytoplankton.

There was a marked difference between the response of the natural populations and *Phaeodactylum*. During August to January PN enrichment did not appreciably stimulate the natural populations, while *Phaeodactylum* showed the highest yield during this period (Fig. 3 and 4). An explanation may be that the natural phytoplankton populations during the summer and autumn are adapted to low concentrations of nutrients. Only during the spring outburst are species adapted to a high supply of nutrients present.

The natural phytoplankton populations on the whole cannot build up as high biomass in the flasks as *Phaeodactylum*. This is probably so because these populations are not adapted to growth in bottles under high light intensities and high temperatures. Some of the species may also have a restricted tolerance towards changes in the nutrient supply, leading to competition with better adapted ones. *Phaeodactylum*, on the other

hand, has a wide ecological range, and no competition is taking place in the single species culture. A consequence of the drastic changes in environmental conditions when phytoplankton are brought from their natural environment to bioassay flasks in the laboratory is a marked change in species composition already after a few days (Table 1). Such changes, from the dominance of one species to another, are virtually unavoidable in bioassay with mixed populations if the experiments are prolonged to more than a few hours (Gerhart and Likens 1975, O'Brien and Denoyelles 1976).

The most obvious increase in the yield of both *Phaeodactylum* and the natural populations occurred when P and N were added together. However, enrichment with a chelator and a trace element mixture further increased the response (Fig. 3 and 4). As these enrichments were not tested separately (except for the initial experiments, Fig. 2) one cannot say if there was an initial growth limitation due to a lack of chelator and/or trace elements, or if this limitation began later as a consequence of the rapid growth when P and N were added. Trace element limitations in bioassay experiments have been recorded, especially by Goldman (1972). Parr and Smith (1976) reported Fe limitation for three algal species grown in Lough Neagh water. Lindahl and Melin (1973) made bioassay tests with brackish water from the polluted Stockholm archipelago. They found no stimulation with chelated trace elements alone or in combination with phosphorus, while the greatest growth was observed with the »complete» addition (P+N+T+E). A stimulation also occurred when N was added alone, which is in agreement with my findings.

Nutrient deficiency has long been recognized as one of the factors that control phytoplankton growth. However, there have been controversies regarding what element(s) is important. For inland waters the debate has focused around phosphorus (Likens 1972), especially in connection with the detergent question. The phytoplankton in coastal waters has, on the other hand, often been considered nitrogen-limited (Ryther and Dunstan 1971, Goldman et al. 1974, Gargas 1975 b, Goldman 1976). Thus phosphate is found in excess — compared to the needs of phytoplankton — in coastal waters, and an addition does not stimulate further growth (cf. Fig. 8 and Ryther and Dunstan 1971). However, it is not always possible to conclude which element limits growth directly from the concentrations of nutrients in water, as the demand of marine algae can vary within wide limits. There can also be a so-called luxury uptake of phosphorus, i.e. phosphorus is stored in the cell and can be used under less favourable conditions. Such a mechanism might explain the unexpectedly small amounts of phosphorus left after the growth of *Phaeodactylum* (Fig. 2).

As phosphorus alone does not stimulate growth in the bioassay experiments, one might draw the conclusion that phosphorus cannot become limiting to phytoplankton growth in the Öresund, and that there is no sense in removing phosphorus from sewage entering this area. However, if P and N are added simultaneously, growth is greatly stimulated. As primary or secondary sewage contains high concentrations of inorganic P and N, such sewage is likely to increase the growth of phytoplankton and benthic algae. If, on the other hand, P is removed from the sewage, N cannot be fully utilized, and phytoplankton growth will be kept down. A removal of nitrogen would of course also be effective (probably more effective than phosphorus removal) but this is not yet economically feasible.

Sammanfattning

Biotestförsök har utförts på vatten från Öresund. Som testorganismer användes dels *Phaeodactylum tricornutum*, dels de vid provtagningstillfällena förekommande naturliga fytoplanktonpopulationerna. Öresundsvatten med testorganismer inkuberades på

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laboratoriet vid $20^{\circ}\text{C} \pm 1$ och ca $4 \cdot 10^{-2} \text{ mW} \cdot \text{cm}^{-2}$ i 500 ml rundkolvar. Vattnet berikades med olika näringslösningar innehållande fosfor, kväve, spårämnen och chelator (EDTA). Tillväxten mättes som cellantal (endast *Phaeodactylum*), klorofyll *a* och ^{14}C -fixering. En god korrelation förelåg mellan cellantal och klorofyllhalt samt mellan klorofyll och ^{14}C -fixering, dock endast om den senare mättes 4 dagar efter försökets start.

Biotesterna gav följande resultat:

1. Fosfortillsats stimulerar ej tillväxten.
2. Kvävetillsats ger en måttlig tillväxt.
3. Kväve och fosfor tillsammans medför kraftig tillväxt.
4. Spårämnen och/eller chelator ökar tillväxten ytterligare något i jämförelse med tillsats av enbart kväve och fosfor.

Försöken bekräftar den slutsats som kan dras ur förhållandet mellan koncentrationerna av fosfor och kväve i Öresund, nämligen att kväve är det primärt begränsande ämnet för alg tillväxt. Trots detta kan en eliminering av fosfor i avloppsvattnet minska den eutrofiering som otvivelaktigt skett och sker i Öresund. En sådan har av vissa forskare (Steemann Nielsen 1976) ansetts vara till fördel t.ex. för fiskproduktionen i Öresund. Det är dock svårt att till alla delar förutse följden av en eutrofiering på hela näringskedjan, från plankton till bottenfauna och fisk. Dessutom måste även de negativa effekterna tas i beaktande, främst en ökande alg tillväxt (både av bentiska alger och fytoplankton), och därmed tilltagande grumlighet i vattnet vilket försämrar Öresunds och Kattegats värde ur rekreationssynpunkt.

Acknowledgements

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INFLUENCE OF SALINITY, PHOSPHORUS AND NITROGEN
ON THE GROWTH OF PHYTOPLANKTON IN THE ÖRESUND.

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ABSTRACT

The growth of natural phytoplankton communities in surface and bottom water from three stations in the Öresund was investigated in laboratory experiments. Mixtures of surface and bottom water were tested, as well as additions of nitrogen and phosphorus. Phosphorus enrichment did not increase phytoplankton growth in surface or bottom water. Nitrogen stimulated phytoplankton growth considerably, and phosphorus plus nitrogen had an even greater effect. Phytoplankton growth in mixtures of surface and bottom water was intermediate to growth in pure surface and bottom water. During a period of one year a total of 41 phytoplankton species were recorded in the bioassay flasks at the end of the experiments. Among these were *Skeletonema costatum*, *Nitzschia closterium* and *Chaetoceros affinis*, species commonly kept in unialgal cultures and often used in experimental work. The laboratory experiments show that Kattegat water generally supports higher phytoplankton biomass than Baltic water, probably owing to its higher nutrient content.

INTRODUCTION

Salinity is considered to be the main factor controlling the distribution of organisms in brackish-water areas. This is probably true for phytoplankton, although many species are euryhaline. Phytoplankton biomass (disregarding species composition) is, on the other hand, mainly a function of light, temperature, nutrient supply and zooplankton grazing.

Along the coasts of Sweden there are excellent opportunities to study the influence of both salinity and nutrients on pelagic primary production and phytoplankton species composition. Strong salinity gradients - both horizontal and vertical - are found in the Öresund between Denmark and Sweden. The Öresund is a shallow channel (max. depth 53 m) and is one of the connections between the Baltic and the Kattegat. The Kattegat continues through the Skagerack into the North Sea (Fig. 1). Although there is a decreasing gradient of salinity from the Skagerack along the Swedish coast up to the Gulf of Bothnia, the strongest change in salinity is found in the Öresund.

The flux of water from the Baltic into the Kattegat means that the Öresund functions as an estuarine area with a salt water wedge penetrating into the deeper layers. Salinity in surface waters of the south part of the Kattegat ranges from 18 to 24⁰/oo, and in the bottom water (below ca 20 m) from 30 to 35⁰/oo (Hermann 1967). In the transition zone between the Baltic and the Öresund salinity usually ranges from 8 to 10⁰/oo. Thus the total range of salinity found in the Öresund is 8-30⁰/oo. For more information about the hydrography of the Öresund, see von Wachenfeldt 1975.

With regard to phytoplankton species composition and biomass, Kattegat water can be characterized as richer than



Fig. 1. Sampling stations in the Öresund.

Baltic water (Edler 1977). Thus the deeper parts of the Öresund are thought to contain potentially more productive water masses than the surface layers. Mixing between the two layers occurs frequently, and it is possible that the generally moderate phytoplankton productivity of surface water in the Öresund can increase after admixture with water from the Kattegat.

In addition to the large salinity variations, the Öresund is also a strongly urbanized area. Sewage from a population of about two million people on the Danish and Swedish sides together with a heavy industrial pollution influence the water (Anon. 1976). There is marked eutrophication, resulting in changes in composition and production of benthic and pelagic algal communities (von Wachenfeldt 1975, Edler 1977). The additional factor of human influence is superimposed on the already complicated environmental conditions caused by salinity variations.

Nutrient limitation of phytoplankton in the surface waters of the Öresund has previously been investigated in bioassay tests using both the natural community and the test alga *Phaeodactylum tricornutum*, Bohlin (Granéli 1978). Similar tests, using the natural phytoplankton community only, were performed in the present investigation on both deep (Kattegat) and surface (Baltic) water from different parts of the Öresund. The main purpose was to investigate differences in nutrient limitation and algal growth potential between the Baltic and Kattegat water.

MATERIALS AND METHODS.

Samples were taken at approximately monthly intervals on 11 occasions from August, 1977, to May, 1978, at three different stations in the Öresund (Fig. 1). Samples were obtained from the surface and near the bottom (minimum distance 5 m from the bottom). The water was poured into 5 l plastic containers and stored in thermally insulated boxes.

The bioassay experiments were started within two hours after sampling. The water was filtered through a 100 μ m nylon net to remove large zooplankton. Three liters was filtered through a Whatmann GF/C filter for initial chlorophyll *a* analyses.

The salinity of the samples was determined with a conductivitymeter. If the salinity in the bottom water (B) was above 18⁰/oo, experiments with water from the surface (S) mixed with bottom water were performed. Two mixtures were used: 90% surface water + 10% bottom water and 50% of each.

Reagent grade chemicals were used to prepare concentrated stock solutions of phosphate and nitrate (KH_2PO_4 and NaNO_3). Small amounts of these solutions, giving final concentrations corresponding to 1% Z8 medium (Skulberg 1964, Gargas and Pedersen 1974), were added to the bioassay flasks. The additions corresponded to final added concentrations of 56 $\mu\text{g PO}_4\text{-P} \cdot \text{l}^{-1}$ and 840 $\mu\text{g NO}_3\text{-N} \cdot \text{l}^{-1}$.

For each station (if the salinity in the bottom sample was above 18⁰/oo) the following series of bioassays was used:

S	=	surface water
S + P	=	" " + phosphorus
S + N	=	" " + nitrogen
S + PN	=	" " + phosphorus + nitrogen
B	=	bottom water
B + P	=	" " + phosphorus
B + N	=	" " + nitrogen
B + PN	=	" " + phosphorus + nitrogen
S + 10% B	=	90% surface water + 10% bottom water
S + 50% B	=	50% " " + 50% " "

The experiments were performed in 500 ml spherical glass flasks containing 400 ml water. The flasks were protected from airborne contamination but sterile working procedures were not followed. Temperature was $20 \pm 1^\circ\text{C}$, and the flasks were exposed to continuous light corresponding to $0.4 \text{ W}\cdot\text{m}^{-2}$ (approx. 4000 lux, Asea-Scandia fluorescent lamps). The cultures were shaken once a day.

Experiments were terminated on the fourth day. Primary production was then measured according to Steemann Nielsen (1952) using a 30 ml subsample incubated for two hours at the same light intensity and temperature as the flasks. Radioactivity of the filters was measured with a GM counter. ^{14}C uptake was transformed to $\text{mg C.m}^{-3}.\text{h}^{-1}$ using pH, temperature, salinity and the formulae in Gargas (1975). Chlorophyll *a* samples on glass-fibre filters were homogenized, and the pigments were extracted in 90% acetone and determined using the trichromatic equations of Strickland and Parsons (1968).

Phytoplankton species were identified (nomenclature according to Hendey, 1974, and Parke and Dixon, 1976) and their abundance determined semiquantitatively at the beginning and end of the experiments using a subjective scale: dominant, subdominant and present. Species composition was analyzed in the following flasks: S + PN, B + PN, S + 10% B and S + 50% B.

Concentrations of inorganic phosphorus ($\text{PO}_4\text{-P}$) and nitrogen ($\text{NO}_3\text{-N} + \text{NO}_2\text{-N} + \text{NH}_4\text{-N}$) in the water on the sampling occasions were obtained from the Swedo-Danish project (unpublished results).

RESULTS.

The most southerly station (no.1, Fig.1) is too shallow, about 15 m, to be influenced by the deep Kattegat water. Hence salinity in the bottom samples reached a maximum of 28 $^{\circ}$ /oo (Fig. 2). On four of the sampling occasions Baltic water (8-10 $^{\circ}$ /oo) was found down to 10 m where the bottom samples were taken.

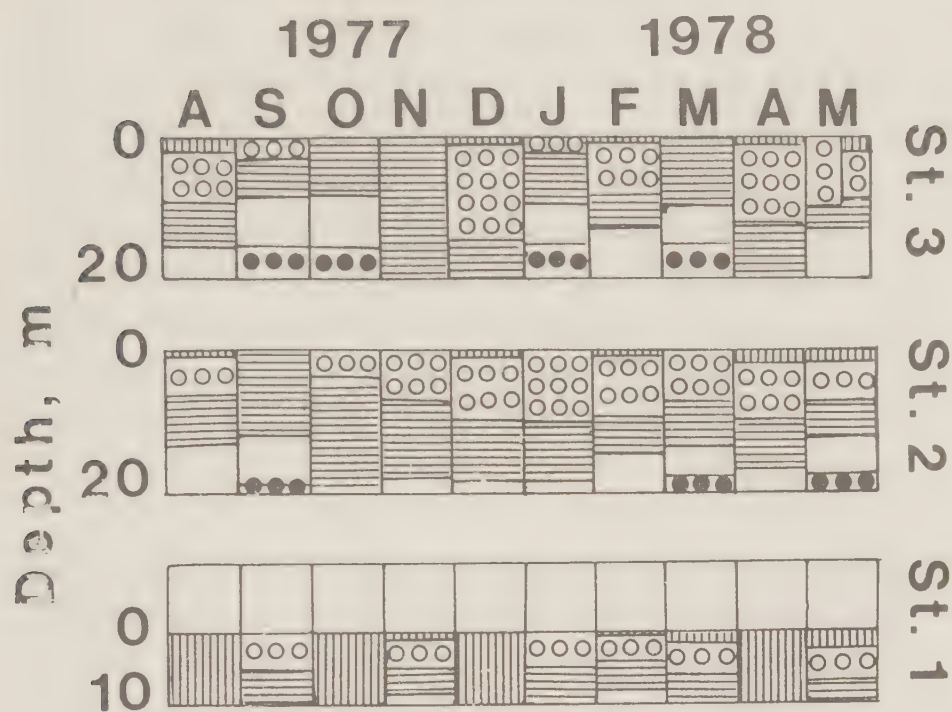
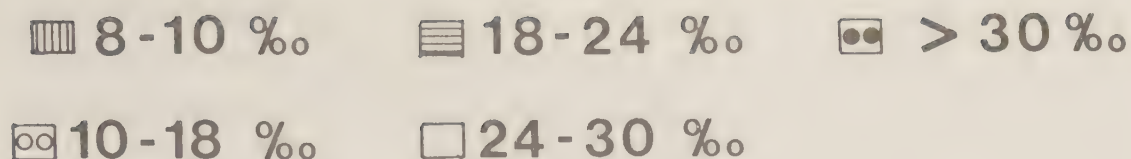


Fig. 2. Salinity stratification during the period of investigation.

At station 2 the depth is considerably greater, about 30 m. Here, bottom samples were taken at 20 m, where Kattegat water was almost always found. On six occasions a mixture of Kattegat surface ($18-24^{\circ}/\text{oo}$) and deep water ($30-35^{\circ}/\text{oo}$) was found, and on four occasions deep water alone. On one occasion surface water extended to 20 m.

The most northerly station (no. 3) is close to the Kattegat. The depth is about 25 m, and salinity conditions resemble those at station 2. At 20 m, where the bottom sample was ta-

ken, Kattegat deep water alone was found on five occasions, a mixture of surface and deep Kattegat water on four occasions, and Kattegat surface water alone on two occasions.

Salinity conditions in surface water from stations 2 and 3 were also similar. Baltic water was found six and five times, Kattegat surface water one and three times, and a mixture of surface and deep Kattegat water four and three times, respectively. Although it is not situated at the southernmost part of the Öresund, station 1 is evidently dominated by Baltic water. At the surface this water was found on eight of the sampling occasions, while the remaining two gave a mixture of Baltic and Kattegat surface water.

TABLE 1. MEAN SALINITIES DURING THE PERIOD AUGUST, 1977, TO MAY, 1978, AT THREE STATIONS IN THE ÖRESUND.

	DEPTH (M)	SALINITY (‰)	N	RANGE
STATION 1	0	9.1	10	7-13
	10	17.9	10	8-28
STATION 2	0	11.3	11	8-18
	20	26.7	11	21-30
STATION 3	0	13.0	11	8-19
	20	27.6	11	21-31

The increasing salinity in the Öresund as one moves northwards is shown by the mean values (Table 1). However, salinity changes rapidly both horizontally and vertically in

the Öresund, and no sharp division between the different waters from the Kattegat and the Baltic occurs because of a continuous mixing of these water masses.

The bottom water at all three stations showed consistently higher concentrations of both inorganic nitrogen and phosphate-phosphorus than the surface water (Fig. 3 and 4). Seasonal variations were rather irregular, but both nitrogen and phosphorus increased during winter in surface water and decreased markedly in spring. This decrease, connected with the spring outburst of phytoplankton at stations 2 and 3, occurred earlier at station 3 than at station 1 and 2. No corresponding trends were seen in the bottom water.

Mean values for both nitrogen and phosphorus for the entire investigation period were roughly two times higher in the bottom water than in the surface water (Table 2). Based on these mean values, N/P quotients have been calculated. These lie in the range 4 to 5 on a weight basis, or 8 to 12 on a molar basis.

Station 1 had the lowest mean values for initial chlorophyll α in both surface and bottom water (Table 3). Chlorophyll values decreased markedly at all stations after the November sampling (Fig. 5). At the most northerly station the spring outburst of phytoplankton was recorded in March, at the middle station in April, but no spring peak in chlorophyll α was seen at the south station where the Baltic water predominates. The bottom water in most cases had a lower chlorophyll concentration than the surface water, which is also evident from the mean values for the stations (Table 3).

The phytoplankton species found in abundance in the water used for bioassay experiments are shown in Table 4. Species diversity increases from station 1 towards station 3. In the

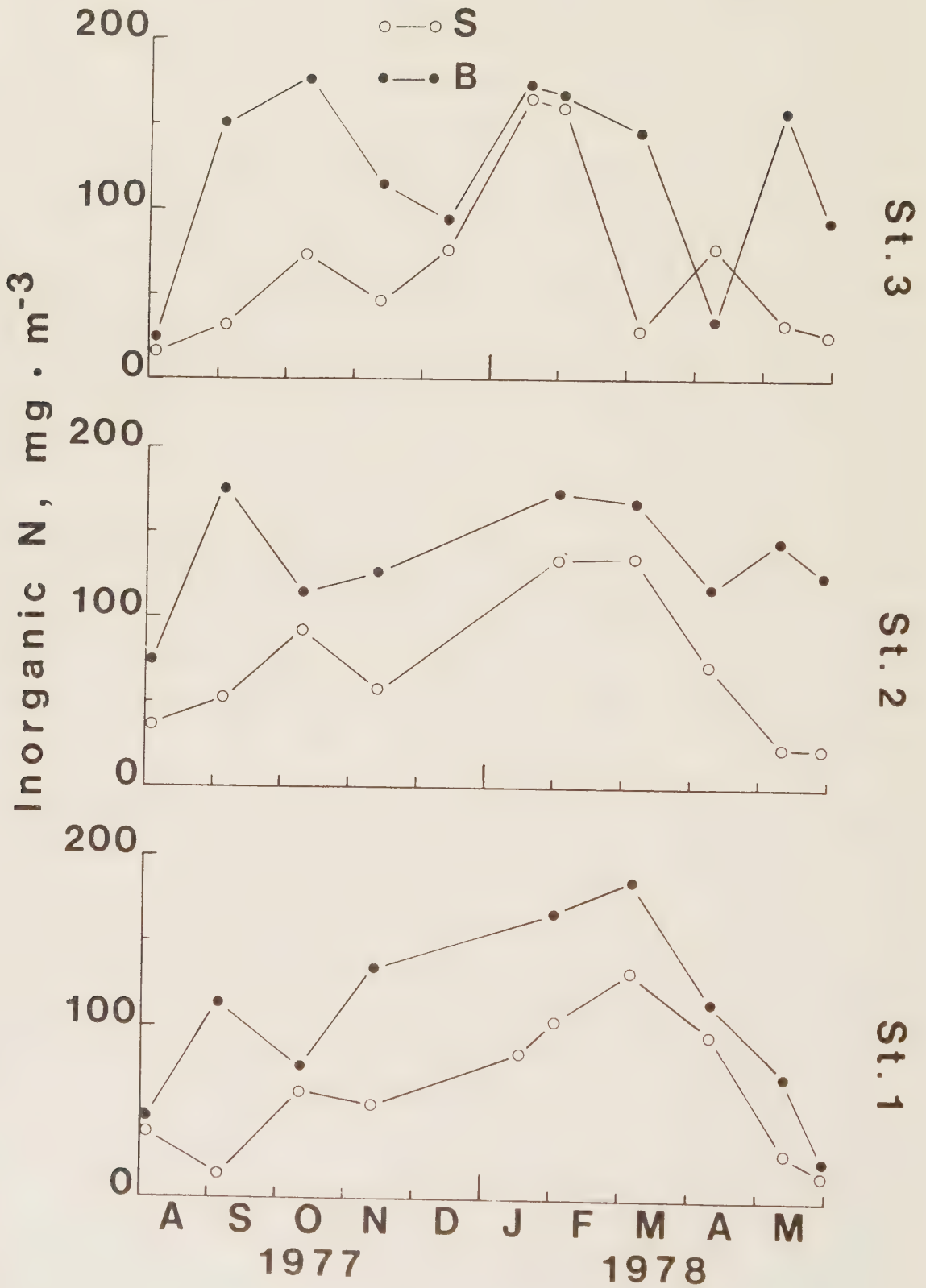


Fig. 3. Concentrations of inorganic nitrogen ($\text{NH}_4\text{-N} + \text{NO}_2\text{-N} + \text{NO}_3\text{-N}$) in surface and bottom water.

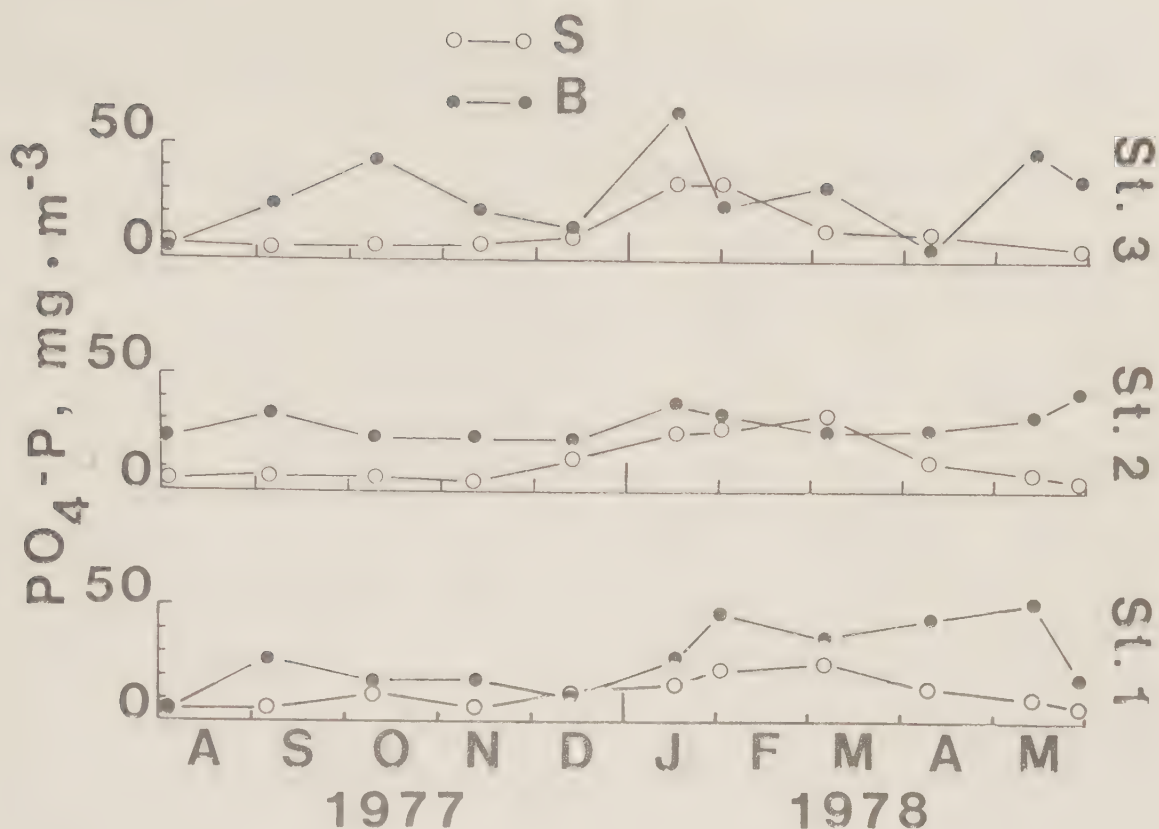


Fig. 4. Concentrations of phosphate ($\text{PO}_4\text{-P}$) in surface and bottom water.

TABLE 2. MEAN CONCENTRATIONS OF INORGANIC NITROGEN ($\text{NO}_3\text{-N} + \text{NO}_2\text{-N} + \text{NH}_4\text{-N}$) AND ORTOPHOSPHATE ($\text{PO}_4\text{-P}$) AT THREE STATIONS IN THE ÖRESUND.

	DEPTH (M)	INORG-N ($\text{MG}\cdot\text{M}^{-3}$)	N	RANGE	$\text{PO}_4\text{-P}$ ($\text{MG}\cdot\text{M}^{-3}$)	N	RANGE	N/P (WEIGHT)
STATION 1	0	63	10	12-130	12	11	5-25	5.25
	10	103	9	20-184	28	11	6-53	3.68
STATION 2	0	70	9	27-136	14	11	4-34	5.00
	20	136	9	74-178	29	11	21-42	4.69
STATION 3	0	67	11	18-164	14	10	6-34	4.79
	20	120	11	22-177	29	11	6-65	4.14

TABLE 3. MEAN INITIAL AND FINAL CHLOROPHYLL a VALUES IN SURFACE AND BOTTOM WATER.

	DEPTH (M)	INITIAL (I) (CHLOROPHYLL a MG·M ⁻³)	FINAL (F) (CHLOROPHYLL a MG·M ⁻³)	F-I	F/I (%)	N
STATION 1	0	1.05	3.22	2.17	307	6
	10	0.84	4.51	3.67	537	6
STATION 2	0	1.80	3.31	1.51	184	11
	20	1.51	5.14	3.63	340	11
STATION 3	0	3.03	3.74	0.71	123	11
	20	2.00	5.56	3.56	278	11

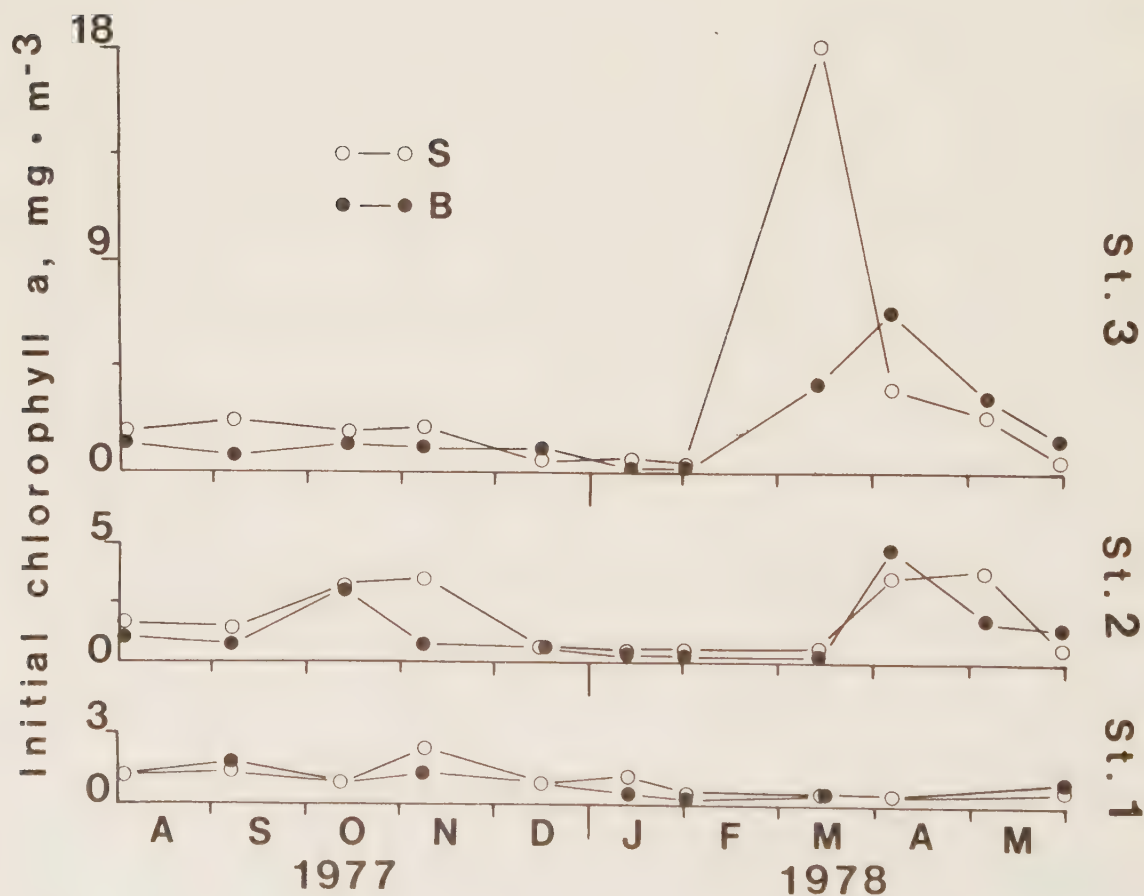
Fig. 5. Chlorophyll a in surface and bottom water at the start of the bioassay experiments.

Table 4. Phytoplankton species found in abundance in the water used for bioassay experiments.

SPRING

Chaetoceros constrictum Gran
 holsaticum Schütt
 perpusillum Cleve
 simile Cleve
 sociale Laud.
 wighamii Brightw.
Detonula confervacea (Cleve) Gran
Euglena sp. Ehrenberg, 1830
Porosira glacialis (Gran) Jörg.
Rhizosolenia hebetata forma *semispina* (Hensen) Gran
Skeletonema costatum (Grev.) Cleve
Thalassiosira nordenskiöldii Cleve

SUMMER

Ceratium lineatum (Ehrenb.) Cleve
Gomphosphaeria spp. Kützinger, 1836
Rhizosolenia alata Brightw.

AUTUMN

Ceratium longipes (Bail.) Gran
 tripos (O. F. Müll.) Gran
Chaetoceros affine Laud.
 constrictum Gran.
 danicum Cleve
 lacinosum Schütt
 radians Schütt
 spp. Ehrenb, 1844
Coscinodiscus granii Gough
Distephanus speculum (Ehrenb.) Haeck
Protoperidinium divergens (Ehrenb.) Balech.
Rhizosolenia fragilissima Berg.
Skeletonema costatum (Grev.) Cleve

WINTER

Aphanizomenon flos-aquae (L.) Ralfs.
Ceratium tripos (O. F. Müll.) Gran
Chaetoceros danicum Cleve
 spp. Ehrenberg, 1844
Coscinodiscus stellaris Roper
Protoperidinium spp. Bergh, 1881
Rhizosolenia alata Brightw.

spring the same species dominated at all stations, although, as can be seen from the chlorophyll a values (Fig. 5), their numbers were quite different.

Final chlorophyll a values in the bioassay experiments are shown in Fig. 6-10. The mean final concentrations of chlorophyll a did not differ greatly among the three stations (Table 5), although the values for station 1 from experiments with S, S + P, B + P, S + 10% B and S + 50% B were slightly lower than corresponding values for station 2. Station 3 had the highest final values in these experiments (except for S + 50% B), as well as the highest initial chlorophyll a values (Table 3). In the experiments with nitrogen addition (S + N and B + N) station 1, on the other hand, showed the highest mean values, while water from station 2 on an average responded more strongly to additions of both nitrogen and phosphorus (S + PN and B + PN) than water from stations 1 and 3.

TABLE 5. MEAN FINAL CONCENTRATIONS OF CHLOROPHYLL a IN THE BIOASSAY FLASKS.

	S	S+P	S+N	S+PN	B	B+P	B+N	B+PN	S+10%B	S+50%B	N
STATION 1 $\text{MG} \cdot \text{M}^{-3}$	3.22	3.28	10.09	22.93	4.51	4.58	15.26	20.02	3.51	3.70	6
%	100	102	313	712	100	102	338	444	105 ¹⁾	90 ¹⁾	6
STATION 2 $\text{MG} \cdot \text{M}^{-3}$	3.31	3.64	8.71	23.27	5.14	5.82	12.47	24.19	4.32	4.74	11
%	100	110	263	703	100	113	243	471	124 ¹⁾	112 ¹⁾	11
STATION 3 $\text{MG} \cdot \text{M}^{-3}$	3.74	3.79	8.42	19.71	5.56	6.08	14.84	18.51	4.39	4.62	11
%	100	101	225	527	100	109	267	333	112 ¹⁾	99 ¹⁾	11
STATION 1-3 $\text{MG} \cdot \text{M}^{-3}$	3.42	3.57	9.07	21.97	5.07	5.49	14.19	20.91	4.07	4.35	3
%	100	104	265	642	100	108	280	412	113 ¹⁾	99 ¹⁾	3

¹⁾ PERCENT OF THEORETICAL VALUE CALCULATED FROM S AND B.

Seasonal variations in final chlorophyll *a* values were irregular for additions of nitrogen and nitrogen plus phosphorus (Fig. 9 and 10). In surface water the highest chlorophyll values after enrichments with nitrogen and phosphorus in combination were found in August, while in bottom water from stations 2 and 3 the peaks appeared later, during autumn and winter. At station 1, PN additions resulted in nearly identical chlorophyll values in surface and bottom water. Here, the lowest values were found at the end of winter with an increase in spring (April).

A general seasonal trend in the final chlorophyll *a* values is more easily seen in the bioassay experiments S, B, S + 10% B, S + 50% B, S + P and B + P (Fig. 6-8). In these treatments final chlorophyll *a* values were always below $10 \text{ mg} \cdot \text{m}^{-3}$, in contrast to additions of nitrogen and nitrogen plus phosphorus, where chlorophyll *a* reached 30 and 60 $\text{mg} \cdot \text{m}^{-3}$, respectively. In the bottles without nitrogen addition, maximum values occurred during November to January in surface and bottom water. Lower values were observed at the beginning and end of the investigation period, i.e. in August and May, and minimum values in February or March.

The average final chlorophyll *a* values for the different stations (Table 5) follow a simple pattern. Enrichment with $56 \mu\text{g PO}_4\text{-P} \cdot \text{l}^{-1}$ increased the biomass of phytoplankton only insignificantly over the values found in the flasks with no additions. The increase was on an average 4% for S and 8% for B. Absolute chlorophyll *a* values were, however, significantly higher for bottom water than for surface water. Thus mean final chlorophyll *a* values in flasks with bottom water without additions or with phosphorus addition only were about 50% higher than in corresponding flasks with surface water. The difference between surface and bottom water was most marked in autumn and early winter, and less pronounced during springs (Figs. 6 and 8).

The addition of $840 \mu\text{g NO}_3\text{-N} \cdot \text{l}^{-1}$ greatly increased chlorophyll *a* concentrations - up to nearly three times that in the controls (S and B) in both surface and bottom water. Bottom water plus nitrogen yielded about 50% higher chlorophyll *a* values on an average than surface water plus nitrogen.

Phosphorus and nitrogen added in combination to surface and bottom water resulted in an even more dramatic increase in the phytoplankton biomass than addition of nitrogen alone. In surface water chlorophyll *a* values increased on an average to more than six times the control values, and in bottom water about four times. The average final yield of phytoplankton biomass was similar for S + PN and B + PN, 22 and 21 $\text{mg chlorophyll } a \cdot \text{m}^{-3}$, respectively. For controls and separate phosphorus and nitrogen additions, bottom water ga-

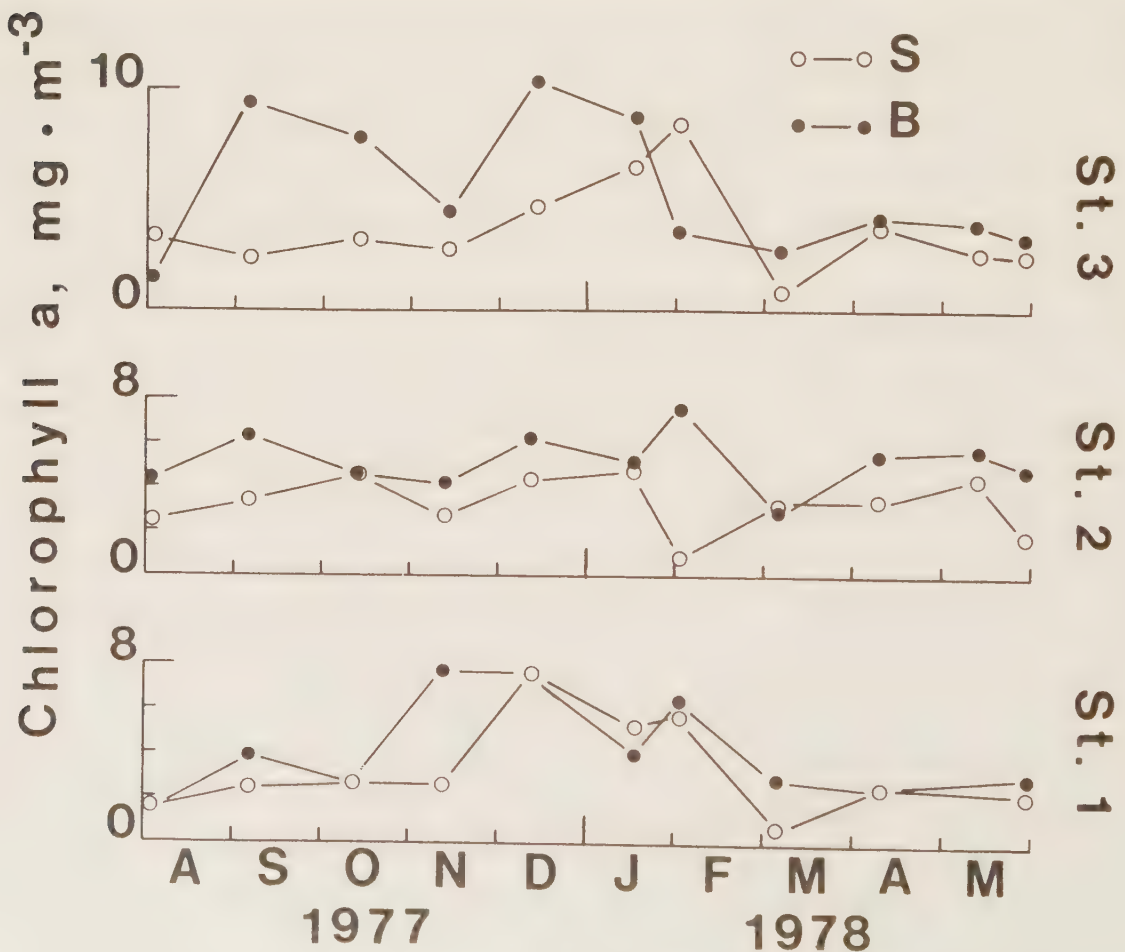


Fig. 6. Final chlorophyll *a* values in the bioassay flasks with only surface and bottom water.

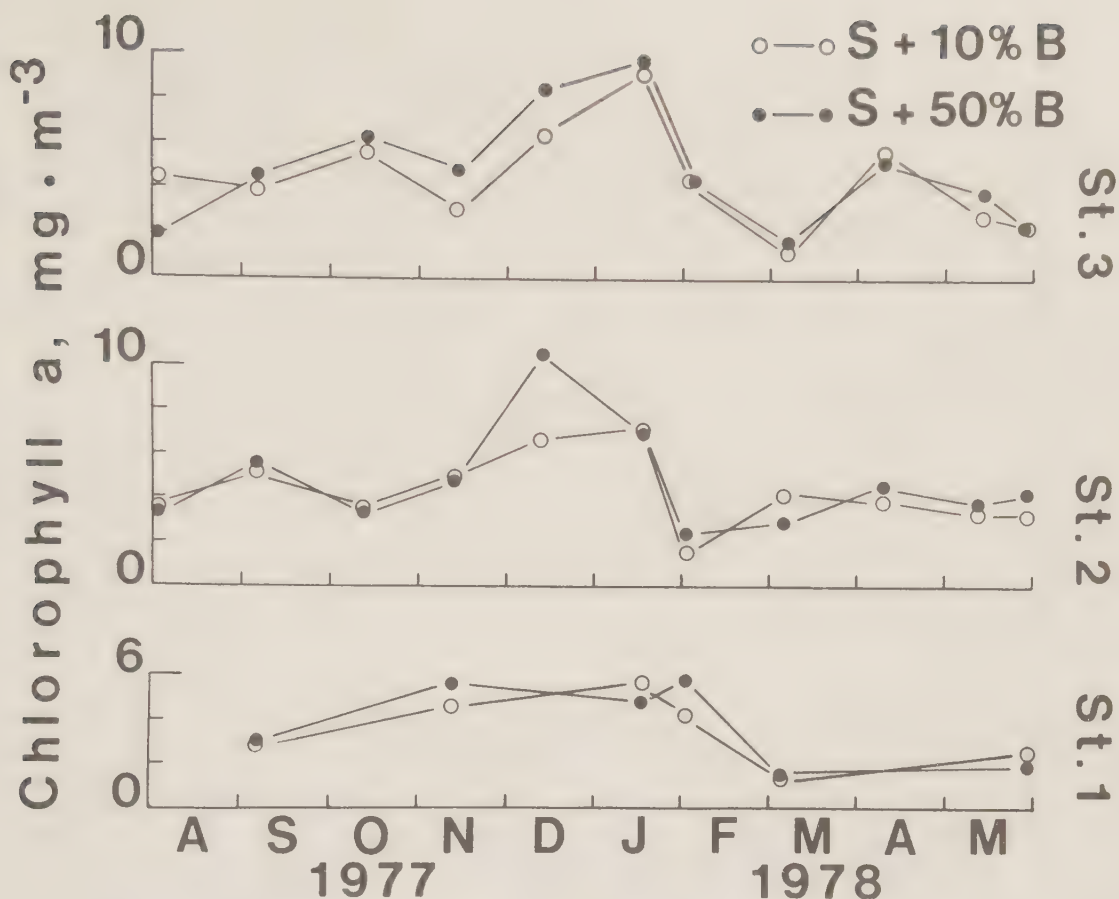


Fig. 7. Final chlorophyll *a* values in the bioassay flasks with mixtures between surface and bottom water

ve about 50% higher chlorophyll *a* concentrations than surface water. This difference between bottom and surface water disappeared when phosphorus and nitrogen were added together.

When surface and bottom water were mixed, the final chlorophyll *a* values were intermediate between values for surface and bottom water alone. The expected final values with 10% addition of bottom water were on average somewhat lower than the measured ones. This "extra" growth was for station one, two and three 5, 24 and 12% of the expected growth, respectively. With 50% addition of bottom water the corresponding difference was on an average -10, 12 and -1%. The seasonal variation in final chlorophyll *a* yield was very similar for both mixtures of bottom water in surface water at the three stations (Fig. 7).

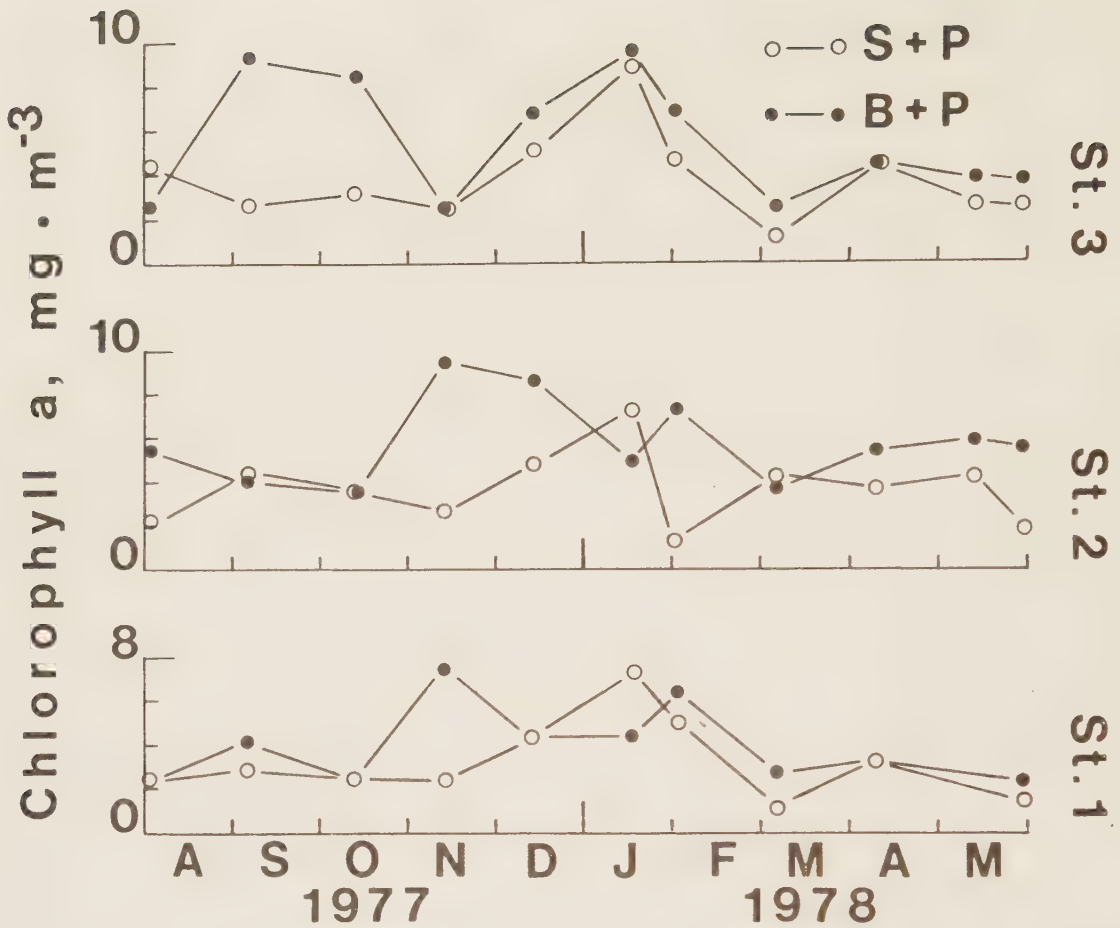


Fig. 8. Final chlorophyll *a* values in the bioassay flasks with phosphorus addition.

Mean final chlorophyll values showed a greater increase over initial values in the flasks with bottom water than in those with surface water (Table 3). Chlorophyll *a* in the surface water from station 1 increased much more, both as a percentage of the initial value and in absolute terms, than in water from station 3; station 2 being intermediate. The absolute increase in bottom water was larger than in surface water and almost the same at all three stations. The percent increases was, however, as in surface water, higher for station 1 than for station 3.

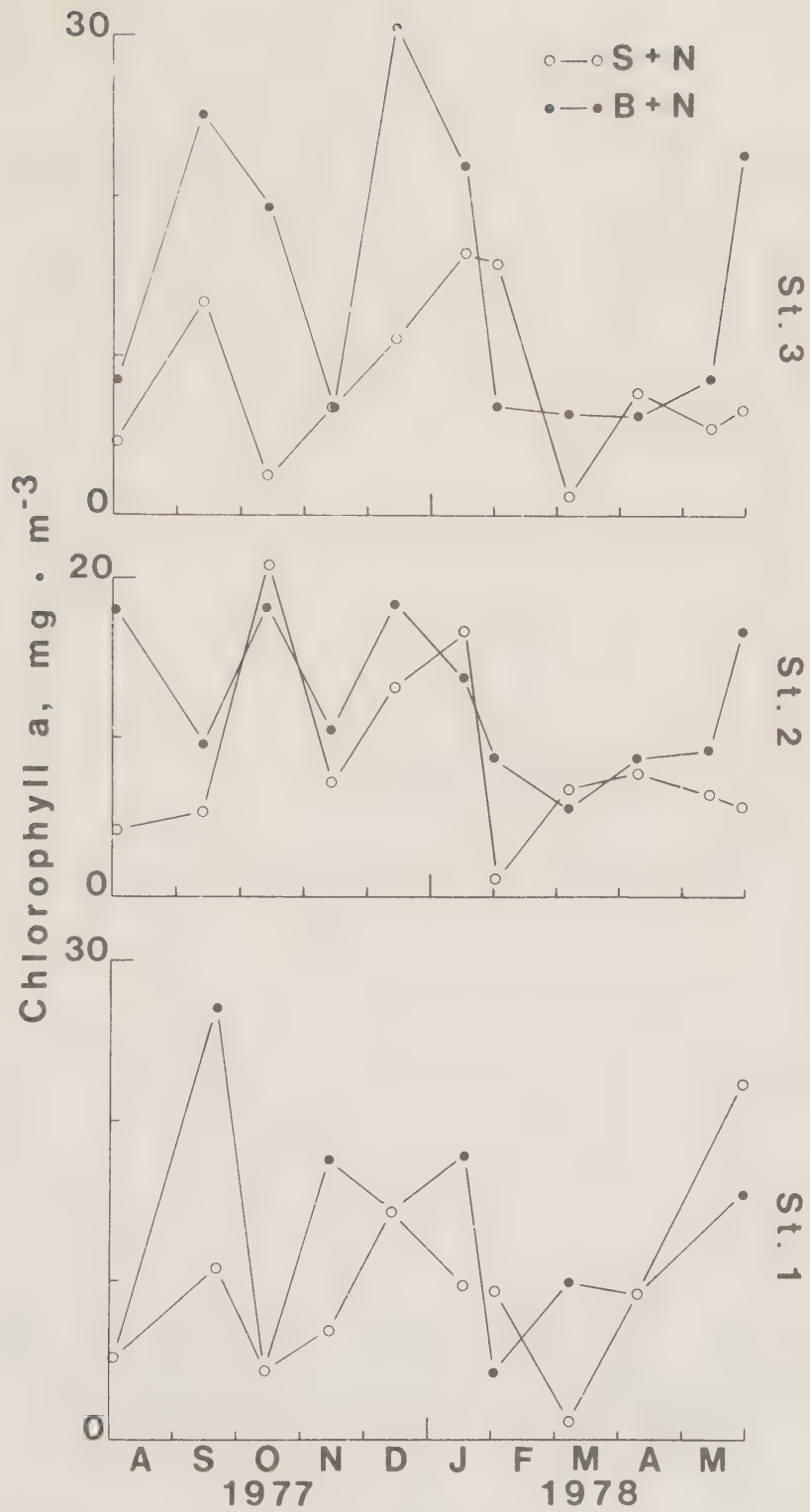


Fig. 9. Final chlorophyll *a* values in the bioassay flasks with nitrogen addition.

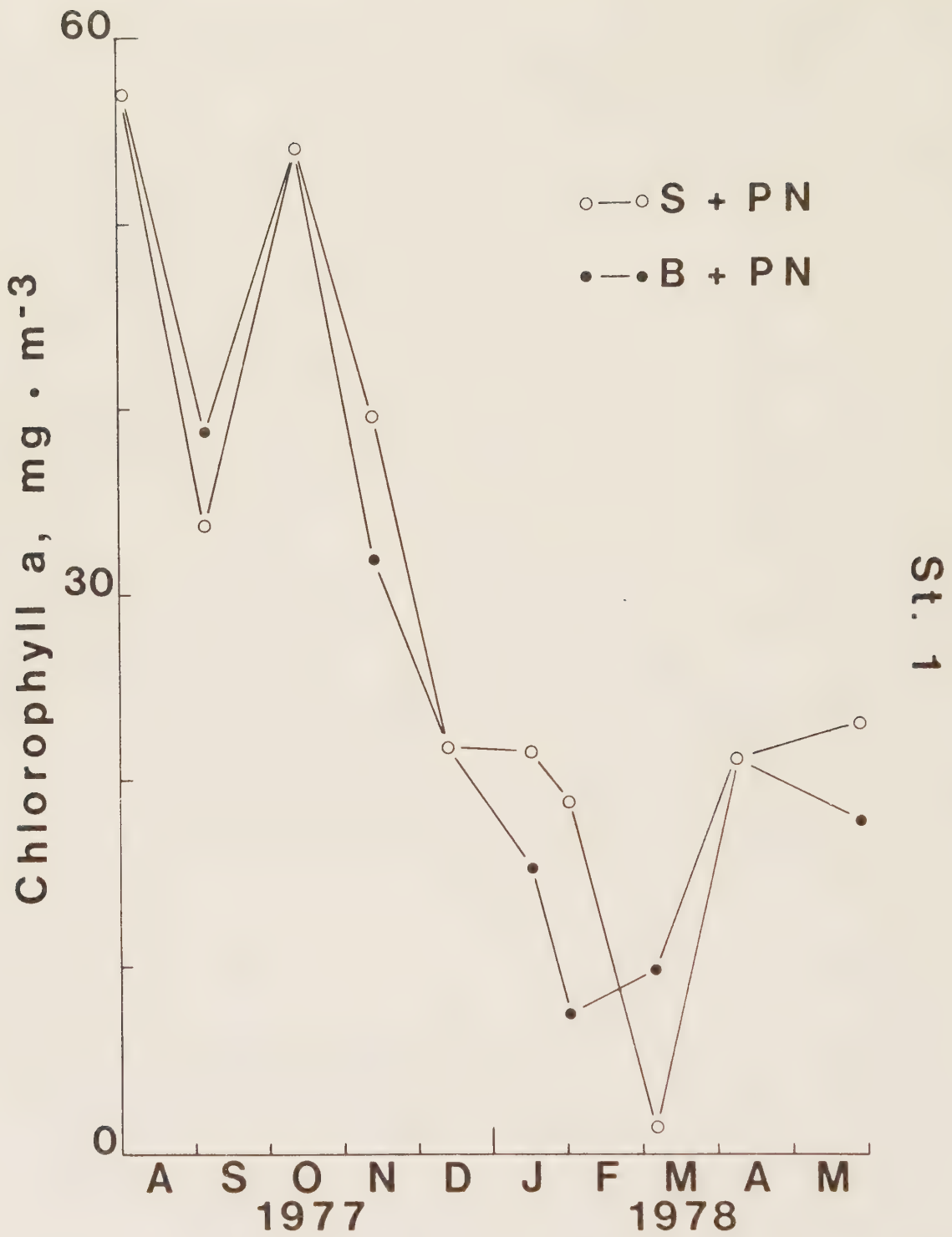
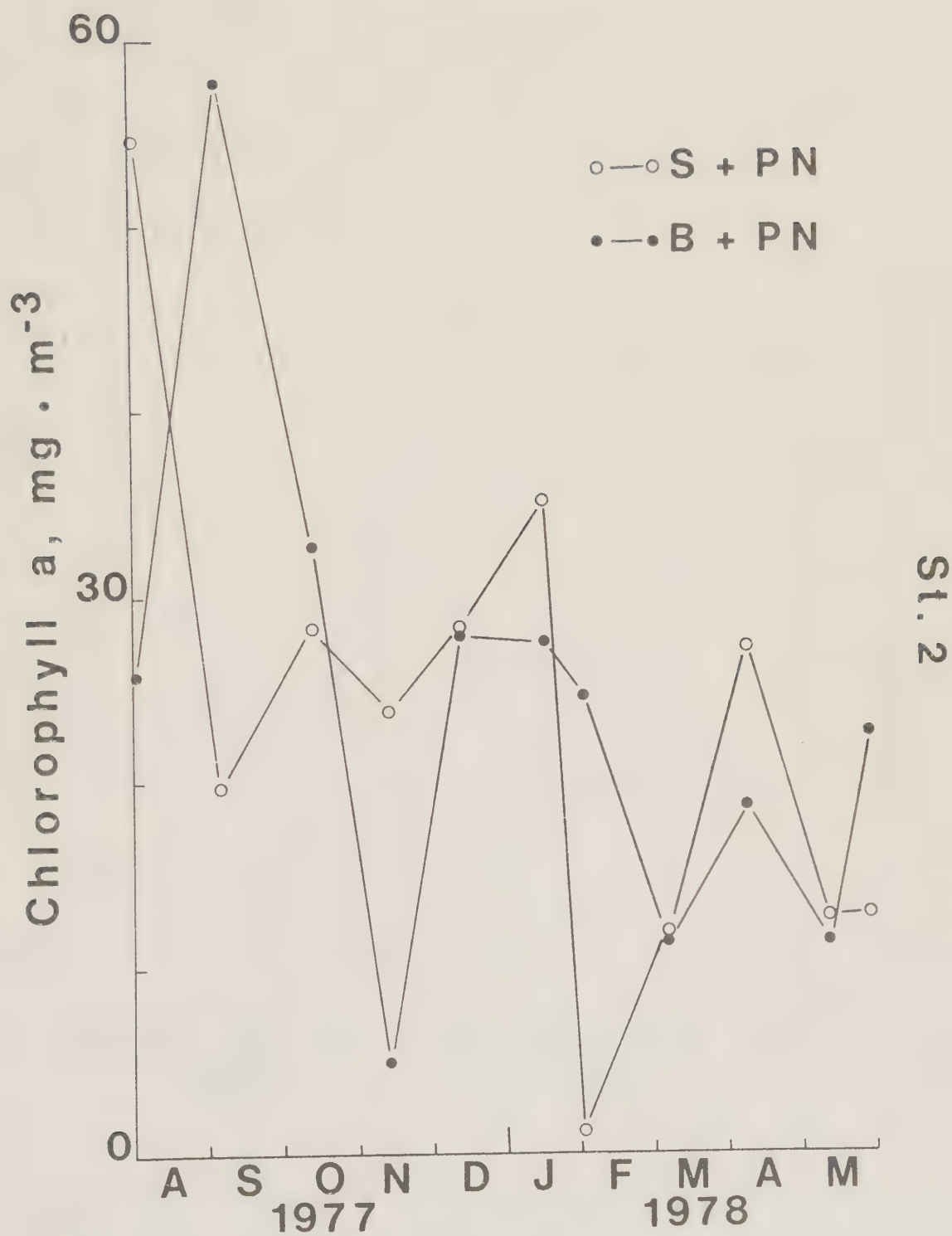
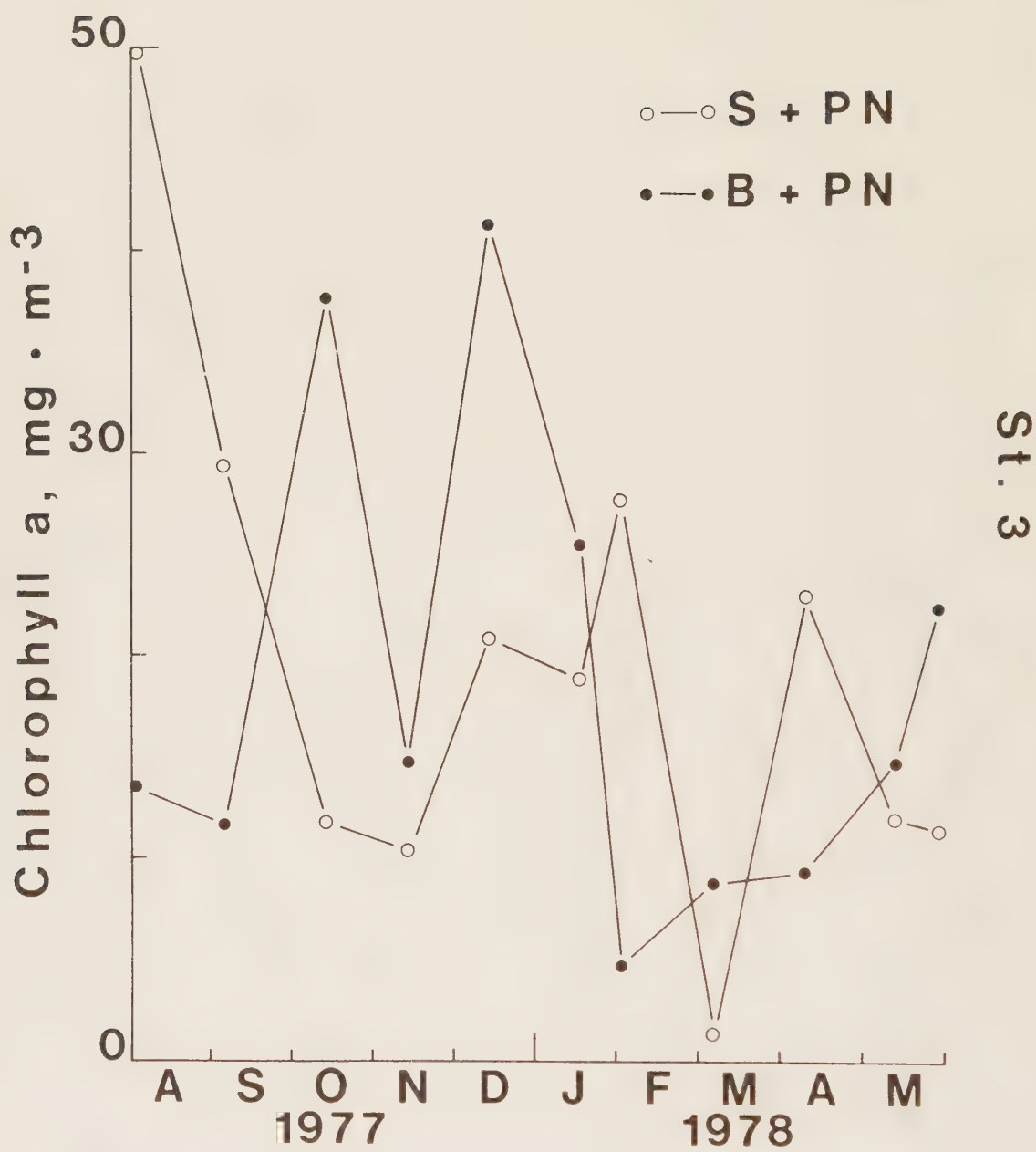


Fig. 10. Final chlorophyll *a* values in the bioassay flasks with phosphorus plus nitrogen addition.





56 phytoplankton species were recorded in the bioassay experiments. The total number initially present probably was much higher as only abundant species were recorded at the start of the incubations. Fifteen of these species, could not be found at the termination of the experiments (Table 6). The remaining 41 species could obviously adapt to conditions in the flasks.

Table 6. Phytoplankton species which disappeared during the four days incubation.

Aphanizomenon flos-aquae (L.) Ralfs.
Ceratium lineatum (Ehrenb.) Cleve
Chaetoceros perpusillum Cleve
 sociale Laud.
Coscinodiscus stellaris Roper
Distephanus speculum (Ehrenb.) Haeck
Euglena sp. Ehrenberg, 1830
Pennata cf. *Achnanthes* spp.
Protoperidinium depressum (Bail.) Balech
 spp. Bergh, 1881
Pyramimonas spp. Schmarda, 1850
Rhizosolenia hebetata forma *semispina* (Hensen) Gran
Thalassionema nitzschoides Hust.
Thalassiosira nordenskiöldii Cleve

If the species recorded at the end of the experiments are grouped according to salinity, only 4 were found exclusively in water with a salinity less than 12⁰/oo (Table 7). The typical Baltic species *Nodularia spumigena* Mert was found in this group. A much larger group (18 species or 44%) contained species only recorded in water with a salinity

higher than 18⁰/oo. 19 species (46%) did not belong to either of the above groups. Among them *Nitzschia closterium* (Ehrenb.) Wm Smith and *Skeletonema costatum* (Grev.) Cleve, two quantitatively important species.

Species recorded in abundance after the fourth day of incubation were *Nitzschia closterium*, *Porosira glacialis* (Gran) Jörg., *Skeletonema costatum* (by far the most abundant), the blue-green algae *Nodularia spumigena* and unidentified monads and flagellates. Another important species was *Rhizosolenia delicatula* Cleve, which never was dominant, however.

DISCUSSION.

Although possible adverse effects of sewage and nutrient additions to the Öresund have caused some controversy (Granéli 1978), the present investigation was not primarily directed towards the problems of eutrophication, but more to consequences of the mixing of two different water masses in this area. It was felt that an investigation using algal bioassay techniques would illuminate the factors controlling phytoplankton production. Edler (1977) studied the phytoplankton in the Öresund during 1972-1973 and included measurements of salinity, temperature, phosphorus, light conditions, phytoplankton succession, biomass and primary production. In his opinion salinity conditions and water transport from the "richer" Kattegat are the most important factors controlling the distribution and production of phytoplankton in the Öresund. Likewise Steemann Nielsen (1937) thought that deepwater phytoplankton in the Öresund originated in surface water of the Kattegat and were brought into the Öresund by the counter current below the north-moving Baltic water. Upwelling of Kattegat water is probably of great importance for phytoplankton in the Öresund, although Edler considers that there is no appreciable addition of nutrients to the surface water through this process.

Table 7. Phytoplankton species found at the end of the bioassay incubations grouped according to salinity preference.

UNDER 12 ‰

Chaetoceros simile Cleve
Blue-green colonies
Nodularia spumigena Mert.
Thalassiosira gravida Cleve

OVER 18 ‰

Ceratium tripos (O. F. Müll.) Gran
Chaetoceros constrictum Gran
 curvisetum Cleve
 danicum Cleve
 didymum Ehrenb.
 externum Gran
 laciniosum Schütt
 septentrionale Oestrup
 subsecundum (Grun) Hust.
Detonula confervacea (Cleve) Gran
Leptocylindrus danicus Cleve
Minuscula bipes (Pauls.) Lebour
Nitzschia longissima (de Bréb) Ralfs ex Pritch
Porosira glacialis (Gran) Jörg.
Prorocentrum micans Ehrenb.
Rhizosolenia alata Brightw.
 pungens Cleve-Euler

BETWEEN 8 AND 33 ‰

Cerataulina pelagica (Cleve) Hendey
Chaetoceros affine Laud.
 ceratosporum Ostenf.
 compressum Laud.
 holsaticum Schütt
 radians Schütt
 wighamii Brightw.
Coscinodiscus granii Gough
 radiatus Ehrenb.
Ditylum brightwellii (West) Grun. ex Van Heurck
Gomphosphaeria spp. Kützinger, 1836
Monads and Flagellates
Nitzschia closterium (Ehrenb.) W. Smith
 pungens Grun.
Oocystis spp. Nägeli in A. Braun, 1855
Prorocentrum micans Ehrenb.
Rhizosolenia delicatula Cleve
 fragilissima Berg.
Skeletonema costatum (Grev.) Cleve

Methods employed for experimental phytoplankton ecology are varied. Use of unialgal, sometimes axenic, cultures is common to test algal growth potential or nutrient limitation. In this type of experiment a restricted number of unicellular or colonial algae are in common use, e.g. *Phaeodactylum tricornutum* and *Skeletonema costatum* for ocean or brackish water. Some of these species are rarely found as dominating constituents of natural phytoplankton communities. Bioassay experiments involving test algae in unialgal cultures have, on the other hand, the great advantage of reproducibility, as the size and physiological status of the inoculum can be carefully controlled. Experiments using the phytoplankton community in the body of water under study obviously have the advantage over experiments using unialgal cultures in that more natural conditions are achieved.

In a previous study of macronutrient and trace element limitation of phytoplankton in the Öresund (Granéli 1978), it was found that batch bioassay tests using both *Phaeodactylum tricornutum* and the natural phytoplankton community gave similar results concerning the general stimulatory effect of different nutrient additions. However, the seasonal patterns with respect to limiting nutrients were different with *Phaeodactylum* and the natural communities.

In the present study batch samples containing the natural phytoplankton community (large zooplankton removed) were kept under continuous light and high temperature for four days. It is clear that not all phytoplankton species survive such conditions. Many are out-competed by a rather limited number of species with a wide ecological breadth, in the present study e.g. *Nitzschia closterium* and *Skeletonema costatum*. Although nutrient limitation can be detected through the type of bioassay used in the present study, only very rough predictions of the effect on phytoplankton of increasing or decreasing nutrient supply to the Öresund can be

made. It has been noted that tests using *Phaeodactylum* and the natural phytoplankton community gave quantitatively similar results in this area. In addition, *in situ* experiments with 100 l plastic bags in the Falsterbo channel south of the Öresund agree well with the results reported here (Granéli and Sundström in prep.).

Growth response was measured as chlorophyll *a* and ^{14}C fixation on the fourth day after the initiation of the bioassay experiments. In the previous study (Granéli 1978) chlorophyll was measured on the 8-10th day and ^{14}C fixation on the fourth day. This study showed that ^{14}C uptake was not stimulated by nutrient additions on the first two days due to a lag phase of phytoplankton growth. On the fourth day, however, the species which could take advantage of added nutrients were in the exponential growth phase and production reached a maximum value. By the 8-10th day these species entered the stationary phase, and the chlorophyll concentration was maximal or even decreasing. When primary production was measured on the fourth day, in the exponential phase, and chlorophyll in the stationary phase, these two parameters were closely correlated (Granéli 1978). In the present study, where both chlorophyll *a* and ^{14}C uptake were measured on the fourth day, the proportionality between the two variables was not so good (Fig. 11). Whereas in the first case $1 \text{ mg chl. } a \cdot \text{m}^{-3}$ was roughly equal to $1 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$, in the current study the relationship was about 1:3. Incubations were made under identical conditions of light and temperature in the two studies. The difference can, of course, be explained by the later chlorophyll determinations in the first study.

The chlorophyll *a* concentration as a measure of phytoplankton biomass was chosen to measure of growth because this is a convenient way to make comparisons with *in situ* concentrations. Primary production, on the other hand, was measured under artificial light conditions and no background values measured in the same manner were available. There is, how-

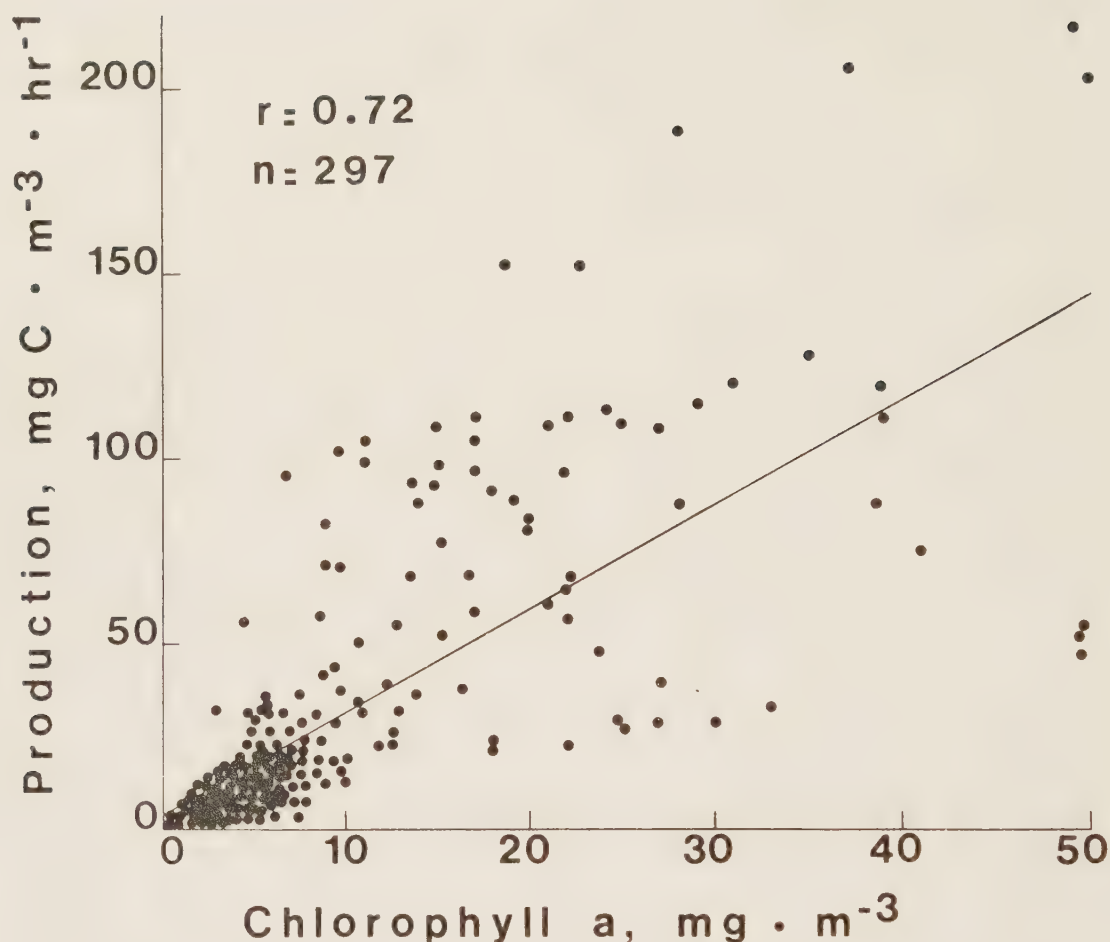


Fig. 11. Relation between final chlorophyll *a* and carbon fixation in the bioassay experiments (after 4 days incubation).

ever, no direct proportionality between phytoplankton biomass measured through cell counting or this value transformed to plasma volume or carbon content and the chlorophyll content of different phytoplankton communities under varying environmental conditions (Edler 1977). In the present study light and temperature were identical for all populations studied, but the chemical composition of the water differed. As chlorophyll synthesis is dependent on nitrogen availability, it is possible that there was a lower biomass/chlorophyll ratio in the flasks with nitrogen addition than in

the other experiments. However, a close correlation between cell numbers of *Phaeodactylum tricornutum* and chlorophyll *a* was observed in experiments similar to the present ones (Granéli 1978).

Brackish water species generally tolerate wide salinity variation (Guilhard and Ryther 1962, Schöne 1974), but they seem to grow better when exposed to low salinities (Nakanishi and Mansi 1965, Qasim et al. 1972). The salinity tolerance of the macrobenthic algae and phytoplankton of the Öresund seems to be quite high, as many of them grow in both the Baltic and in the Kattegat, i.e. a salinity range from less than 10⁰/oo to over 30⁰/oo (von Wachenfeldt 1975, Edler 1977). Although the low salinity in the Baltic water may exclude some species (in Table 4 diversity appears to be substantially lower at station 1 than station 3) found in the Kattegat water, this does not automatically imply a higher biomass in the more saline environment. It is a well known fact that under extreme environmental conditions a few species may produce as high biomass as a greater number of species under "normal" conditions. Thus the "richness" of the Kattegat water applies to number of species but not necessarily to biomass.

Unfortunately, it is not easy to isolate the effects of salinity on phytoplankton biomass and production in the Öresund from other environmental factors, as the different water masses differ not only in salinity but also in nutrient content. Salinity differences are most pronounced vertically, but there is also an increase, both for surface and bottom water, when moving from station 1 to station 3 (Table 1). However, with the exception of bottom water from station 1, the changes are small when mean values for the investigation period are considered. The low salinity in bottom water from station 1 is explained by the fact that this station is so shallow that bottom water was taken at a depth of only 10 m

compared to 20 m at the other stations. Judging from the mean values, an increase in inorganic nitrogen and phosphorus corresponding to the salinity gradient from south to north does not exist (Table 2). The difference in nutrient concentrations between the stations is not statistically significant.

Even if salinity and macronutrients did not vary greatly among the stations, the mean initial chlorophyll values were substantially higher at station 3 than at station 1 (Table 3). It is not possible from the available information to determine what factor caused this difference. One may speculate that mixing of the north-going Baltic water with Kattegat water, which mainly occurs in the south part of the Öresund, increases nutrient availability in the surface water, as deep Kattegat water has a higher nutrient content than water from the Baltic. During transport northwards there can be a build-up of higher chlorophyll levels because of favourable light conditions in the surface water.

Levels of inorganic nitrogen and phosphorus are about twice as high in deep water as in surface water (Table 2). Thus an upwelling would result in an appreciable fertilization of the surface waters of the Öresund. This fertilizing ability of the bottom water is seen in the final chlorophyll values in the bioassay flasks without additions (Table 3). In spite of higher initial values in the S flasks, final values were significantly higher in B flasks in nearly all cases (Fig. 5 and 6).

Growth in the flasks with surface and bottom water without enrichment may thus be proportional to initial nutrient levels. As phosphorus did not stimulate growth, it seems unlikely that the chlorophyll increase in flasks without enrichment should be proportional to initial phosphorus concentrations. Phosphate-phosphorus and growth (mea-

sured as the difference between final and initial chlorophyll values) were not correlated, while there was a significant correlation between inorganic nitrogen and growth (Fig. 12).

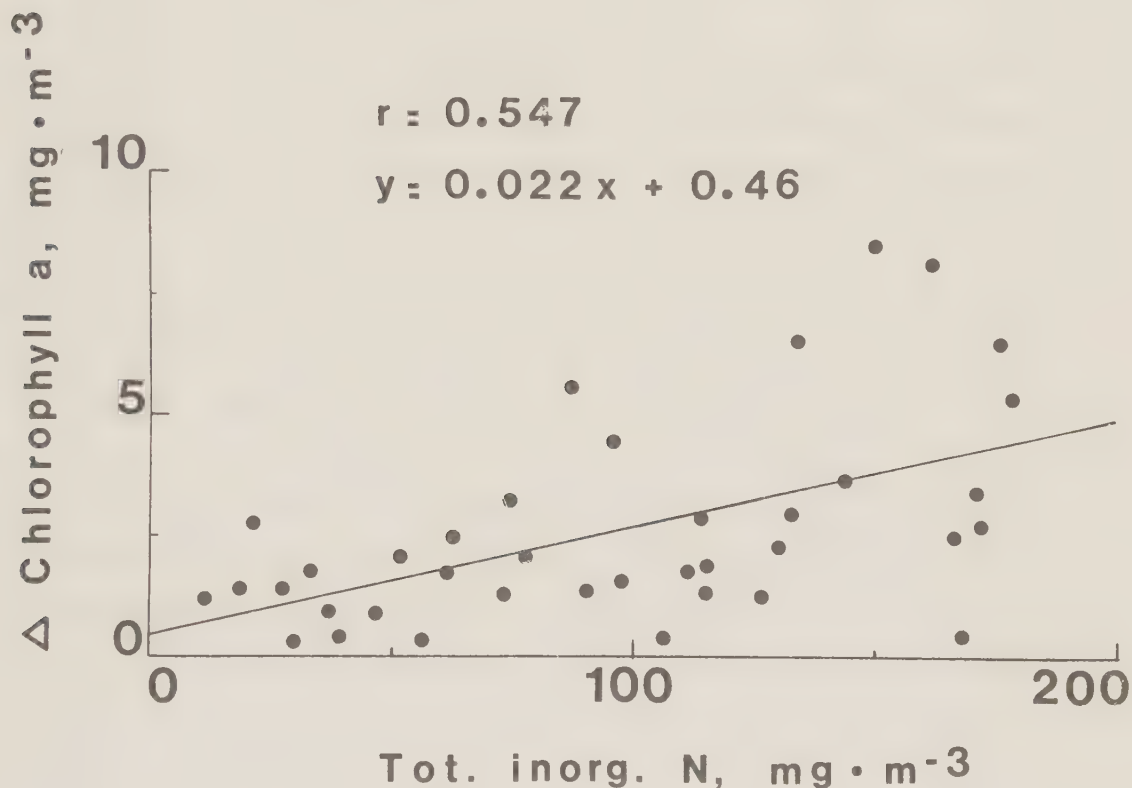


Fig. 12. Relation between concentration of inorganic nitrogen at the start of the bioassay experiments and phytoplankton growth measured as the difference between final and initial chlorophyll *a* concentrations (S and B).

While phosphorus is considered to be the most limiting nutrient in most poorly to moderately productive fresh waters (Shapiro 1970, Schindler 1977), nitrogen deficiency often seems to limit phytoplankton growth in both unproductive

and productive marine areas (Ryther and Dunstan 1971, Goldman et al. 1973). Although the Baltic receives fresh water in such amounts that salinity in the surface water is less than 10⁰/oo, nitrogen deficiency seems to be more important than phosphorus limitation (France and Nehring 1971, Sen Gupta 1972, 1973, Granéli 1978).

With respect to reactions to phosphorus and nitrogen enrichment, there do not seem to be any great differences between the phytoplankton in surface and bottom water (Table 5). Phosphorus additions were rarely significantly stimulating in comparison to samples without enrichment. The only exception was in the beginning of August when phosphorus additions to surface water from stations 1 and 3 increased the chlorophyll concentration somewhat. On this occasion the blue-green algae *Nodularia spumigena* was found in abundance (cf. Granéli 1978). This algae can fix molecular nitrogen (Rinne et al. 1977, Rinne and Tarkiainen 1978), a process which for many heterocystforming cyanophytes is stimulated by high phosphorus concentrations but inhibited by combined nitrogen (Walmsley and Toerien 1975, Lundgren 1976, Granéli and Sundström in prep.). *N. spumigena* is a typical brackish water species which sometimes appears as blooms over vast areas of the Baltic (Horstmann 1975, Öström 1976, Niemi 1976).

Although phosphorus enrichment gave a mean stimulation of a few percent (Table 5), there were several occasions when phosphorus additions actually depressed chlorophyll values. Such an inhibition has also been observed in experiments with plastic bags exposed *in situ*, but no explanation has been found.

Judging from ratios of inorganic nitrogen to phosphorus, nitrogen should be the element which first becomes limiting to phytoplankton growth in the Öresund. The mean ratio was

about 5 on a weight basis (11 on a molar basis) for surface water and about 4 (9) for bottom water (Table 2). This is somewhat below the commonly cited optimum ratio of about 7 (15) (Redfield 1958, Ryther and Dunstan 1971).

Stimulation of chlorophyll synthesis in all nitrogen-enriched bioassay flasks is easily seen in both the mean values (Table 5) and the seasonal variations (Fig. 9). The initial inorganic nitrogen concentration in the samples without enrichment is correlated with the chlorophyll increase (Fig. 12). The average difference in final chlorophyll concentration between surface and bottom water (about $5 \text{ mg} \cdot \text{m}^{-3}$) after nitrogen addition can be explained by the higher phosphorus concentration in the bottom water, the difference being about $15 \text{ mg PO}_4\text{-P} \cdot \text{m}^{-3}$. Because of the high nitrogen addition in the experiments ($840 \text{ mg NO}_3\text{-N} \cdot \text{m}^{-3}$), it is less likely that the initial difference in nitrogen concentration (about $50 \text{ mg N} \cdot \text{m}^{-3}$) could be responsible for the higher chlorophyll values in the bottom samples. Of course, other differences between surface and bottom water might have been involved, e.g. trace elements and chelators which increased the growth of both *Phaeodactylum tricornutum* and the natural phytoplankton community cultivated in surface water from the Öresund (Granéli 1978).

When phosphorus and nitrogen were added together, surface and bottom water produced about the same amounts of chlorophyll (Table 5). Hence, there is no significant difference between the algal growth potential of surface and bottom water once phosphorus and nitrogen limitation has been overcome.

The most obvious difference between surface and bottom water is, of course, salinity (mean difference of 15‰ between 0 m and 20 m). The bioassay experiments show, that this large difference does not influence the potential ability of the two water masses to support phytoplankton growth.

One of the main objectives of the present investigation was to test additions of bottom water for stimulatory effect on the biomass and production of phytoplankton in the surface water of the Öresund. This stimulation was found (Table 5). As the increase in chlorophyll (calculated from the final values for pure surface and pure bottom water), is close to what could be expected, it seems reasonable to ascribe the stimulation to the higher nitrogen content in the bottom water.

In bioassay experiments which involve drastic changes in light, temperature and nutrient concentrations compared to conditions *in situ*, only a limited number of species usually survive. These species often multiply rapidly and are mostly diatoms, which seem to be best adapted to take advantage of the increased nutrient concentrations in enrichment cultures of marine or brackish waters. In the Öresund these diatom species belonged to the genera *Rhizosolenia*, *Skeletonema* and *Nitzschia* (Granéli 1978). As was noted above, the blue-green alga *Nodularia spumigena* was stimulated by increased phosphorus concentrations in late summer experiments. During the summer monads can grow vigorously after a nutrient addition.

Skeletonema costatum, which generally dominated at the end of the bioassay experiments, is common in many areas. In the Öresund it appears both during the spring and autumn, dominating during the latter period according to Edler (1977). *Rhizosolenia hebetata* forma *semispina* (Hensen) Gran is another diatomic species which Edler feels is brought into the Öresund from the Kattegat. Contrary to *Skeletonema costatum*, it did not survive in the bioassay flasks. *Thalassionema nitzschoides* Hust. is characterized as an all-year-round species by Edler, but in spite of this, it did not survive incubation in the bioassay flasks for four days.

Because the survival and development of many of the species initially present in the bioassay samples obviously depend on experimental conditions, it is difficult to transfer the laboratory results to the natural system. Inoculum size and species composition did not exert any decisive influence on the final biomass in the bioassay flasks; but, under natural conditions, with much lower light and temperature levels, phytoplankton are probably dividing slower (cf., however, Eppley 1972). Because of the short renewal time of the water masses in the Öresund (2-3 days for 0-10 m, 12 days for 15-20 m according to Edler, 1977), it is possible that an addition of e.g. nitrate to surface waters would not affect phytoplankton until they have been transported out of the Öresund.

The most important conclusion from the investigation is that the higher algal growth potential of bottom (Kattegat) water in the Öresund is mainly caused by higher inorganic nitrogen concentrations and not by this water's higher salinity and richer flora.

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IN SITU NUTRIENT BIOASSAY IN THE FALSTERBO CHANNEL.
NUTRIENTS ADDED AT THE BEGINNING OF THE EXPERIMENTS

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ABSTRACT

In situ nutrient enrichment experiments were performed during the summers of 1978 and 1979 in the Falsterbo Channel at the south entrance to the Öresund, SW Sweden. Polyethylene bags with 150 l unfiltered surface water were enriched with phosphate ($55-110 \text{ mg P} \cdot \text{m}^{-3}$) alone, or in combination with ammonia or nitrate ($820-1900 \text{ mg N} \cdot \text{m}^{-3}$). When the experiments were started nutrient and chlorophyll *a* concentrations and inorganic N/P weight ratios were low in the channel ($\text{PO}_4\text{-P} < 15$, $\text{NH}_4\text{-N} < 20$, $\text{NO}_3\text{-N} < 5$, chl *a* $< 2 \text{ mg} \cdot \text{m}^{-3}$, N/P 1-3).

Phytoplankton biomass and production and nutrient concentrations in the bags were measured daily for 8 - 10 days after enrichment. Chlorophyll *a* and primary production (^{14}C fixation) increased in all bags compared to initial values. Phosphate enrichment alone did not increase phytoplankton growth over that of the control bag. Nitrate or ammonia alone were stimulatory, while simultaneous addition of both phosphate and ammonia increased phytoplankton biomass considerably (maximum about $50 \text{ mg chl } a \cdot \text{m}^{-3}$).

Assimilation numbers were somewhat higher in bags enriched with nitrogen or nitrogen plus phosphorus compared to unenriched bags. There was no systematic difference in growth rate or biomass yield for ammonia-enriched bags compared to nitrate-enriched bags. In a phosphate-enriched bag POP/ chl *a* increased to over 10, while in the corresponding control bag the mean quotient was under 1. In bags enriched with nitrate, ammonia or either of these together with phosphate, POP/chl *a* quotients were under 2 at the time of the chlorophyll *a* maxima (6 to 9 days after enrichment). In one experiment, phosphorus in bags without phosphate addition (about $20 \text{ mg tot-P} \cdot \text{m}^{-3}$) was mainly in the form of DOP (about $15 \text{ mg P} \cdot \text{m}^{-3}$), with very little POP. In a single experiment, POC/chl *a* quotients were initially

ca 180 in all bags, indicating a substantial detrital fraction of the total seston and/or slow phytoplankton growth rate. Values decreased considerably in all bags after the second day and reached 50 - 70 at the time of the chlorophyll *a* maxima. From this and previous investigations it is concluded that potential phytoplankton biomass and possibly growth rate are nitrogen-limited in the Öresund and adjacent waters

INTRODUCTION

Nutrient limitation of growth rate or biomass yield in algal communities has been a subject of great interest for marine biologists and limnologists. This is because primary production in aquatic environments is thought to be controlled to a large extent by the availability of nitrogen, phosphorus, silica, iron and other elements or compounds. If production of phytoplankton is nutrient-limited, so is ultimately fish production. Thus nutrient availability and commercial fish catches are linked (cfr Ryther 1969). During the last two decades an increasing amount of research has been devoted to the problem of cultural eutrophication. Here, too, research on nutrient limitation of phytoplankton growth plays a dominant role.

Algae - nutrient relationships have been investigated through analyses of nutrient concentrations in water and composition of phytoplankton (e.g. Sen Gupta and Koroleff 1973). A more direct approach involves measurement of nutrient uptake kinetics using isotope techniques (Harrison et al. 1977, McCarthy and Eppley 1972). Both natural phytoplankton communities in shipboard experiments (Eppley et al. 1977) and monocultures in chemostats (Conway 1977) have been used. The nutrient status of algal cells can also be investigated through the activity of specific enzyme systems, e. g. alkaline phosphatase (Pettersson 1980).

Another approach to investigations of nutrient limitation in phytoplankton is enrichment experiments (Gerhart and Likens 1975). Single species cultures, multispecies cultures and natural phytoplankton communities have been utilized. Experiments using specific test algae are often performed in bottles or test tubes in the laboratory. Many marine biologists and limnologists have preferred to work with enclosures *in situ* (bags, cylinders etc), however,

which delimit variously-sized volumes of the plankton community (Brockmann et al. 1974, Menzel and Case 1977, Leah 1978).

Laboratory enrichment bioassays using both unialgal cultures (*Phaeodactylum tricornutum* Bohlin) and natural phytoplankton communities were conducted during 1976-77 with water from the Öresund in an attempt to identify the nutrient potentially limiting phytoplankton production (Granéli 1978). Nitrogen additions had a stimulating effect while phosphorus additions generally did not increase phytoplankton growth.

During 1977-78 similar bottle enrichment experiments were done with the natural phytoplankton communities using water from different stations and depths in the Öresund (Granéli 1980). The principal aim was to investigate effects of the more saline and nutrient-rich Öresund deep water (derived from the Kattegat) on the less-saline and nutrient-poor Öresund surface water (derived from the Baltic). It was shown that Öresund deep water could stimulate phytoplankton growth more than Baltic water, and that nitrogen in both cases seemed to be the most limiting nutrient.

The bioassay experiments discussed in the present article were performed during the summers of 1978 and 1979. Plastic bags of 200 l volume were filled with water containing the natural plankton communities. After initial nutrient enrichment the bags were exposed *in situ* in the Falsterbo Channel at the south entrance to the Öresund. The main objectives of the experiments were:

1. To check if conclusions drawn from previous laboratory experiments with phosphorus and nitrogen enrichment were also valid for *in situ* experiments.

2. To investigate nutrient limitation in South Baltic water. Previous experiments were made with Öresund water, which is influenced by sewage discharge. Nutrient levels at the south entrance to the Öresund are significantly lower than in central Öresund and represent conditions in the South Baltic.
3. To observe any differences in the uptake and effects on phytoplankton of ammonia and nitrate. Earlier laboratory experiments were made exclusively with nitrate additions.

Phosphorus additions normally do not increase phytoplankton production or biomass in Öresund waters. However, added phosphate is probably incorporated into organisms. To investigate this, different phosphorus fractions were analyzed in one of the four bag experiments. Chlorophyll *a* and phytoplankton biomass might not be proportional in this kind of experiment (Granéli in press). As an additional measure of response of the plankton community to nutrient additions, POC was analyzed in one experiment.

MATERIAL AND METHODS.

Enrichment experiments were performed in the widened south entrance to the Falsterbo Channel, at the southwestern tip of Scania in south Sweden (Fig 1). A laboratory belonging to the National Swedish Environment Protection Board, where most of the analyses were made, is situated close to the experimental site.

In 1978 three experiments were carried out on the following dates: (a) June 27 to July 4, (b) August 3 to 11, (c) August 5 to 14 and in 1979 one experiment: (d) June 23 to July 1 (Tab 1).

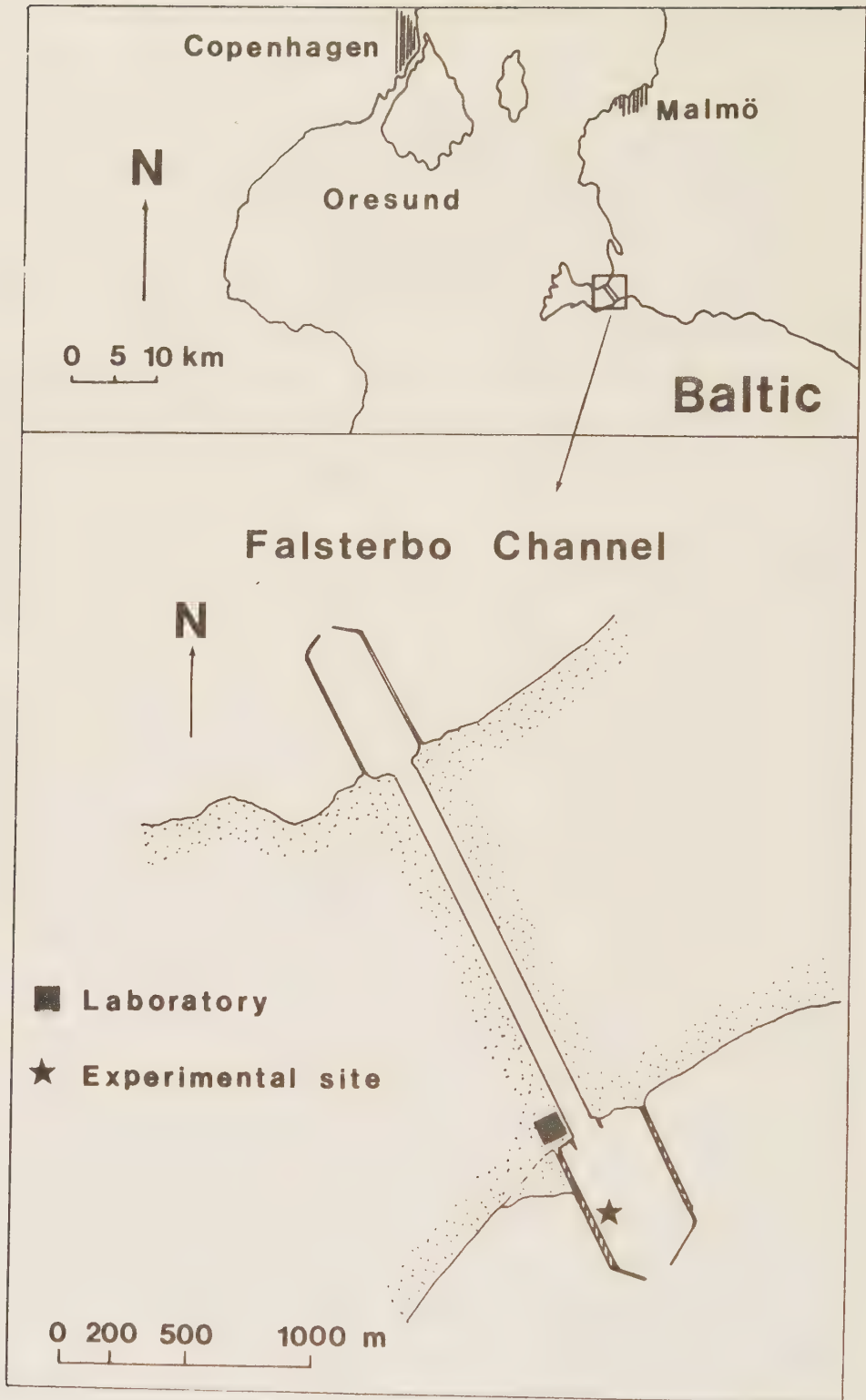


Fig 1. Location of the Falsterbo Channel at the south entrance to the Öresund.

Double polyethylene bags of 200 l volume (800x1300x0.10 mm) were fixed to a wooden frame supported by styrofoam floats at each end (Fig 2). 5 or 6 double bags were nailed to each frame with openings about 30 cm above water level. The construction was anchored at one end only at about 4 m water depth. Thus the frames could move freely with wind and wave motions. Although the entrance to the channel is protected from external wave action, disturbance from boats of up to 15 000 tons is very frequent during the summer months. As droppings from sea gulls often interfere with this kind of experimental set-up, the frames were equipped with a roof made from 0.10 mm polyethylene. Free passage of air was possible over the bag openings, however. Both roof and bags were made from transparent material.

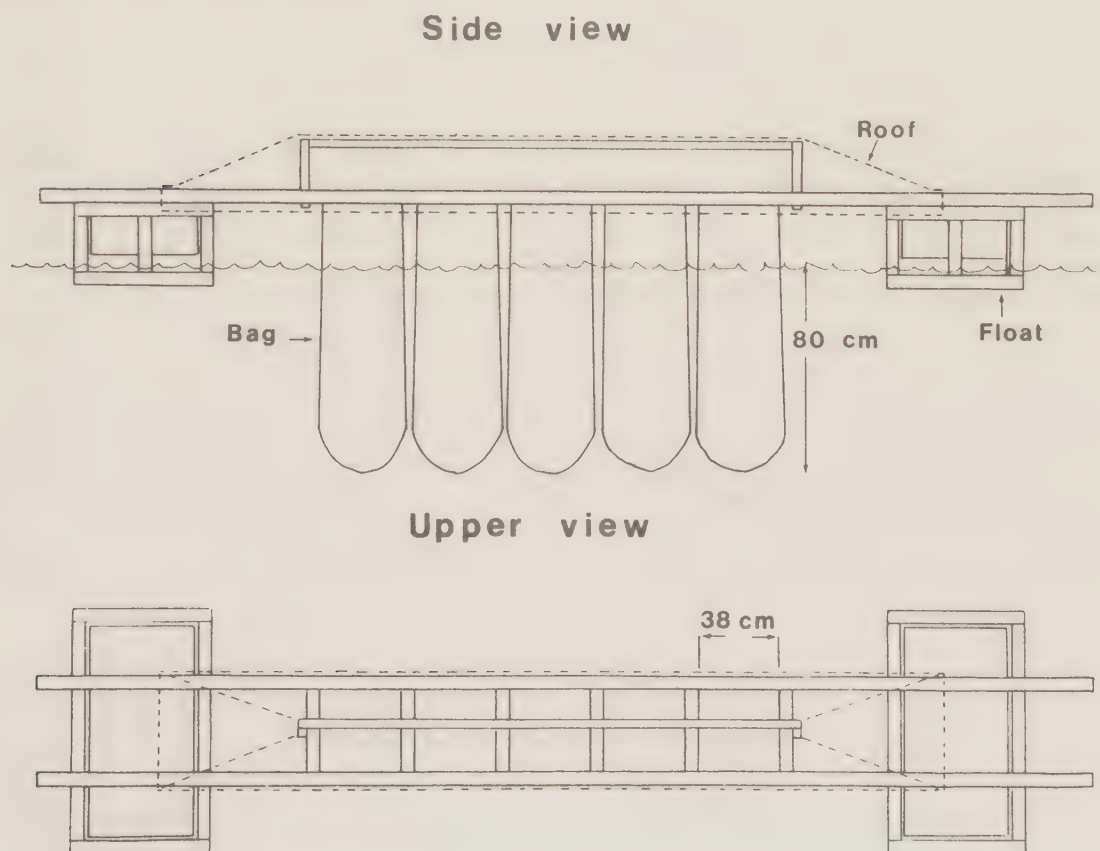


Fig 2. Construction used for the suspension of plastic bags *in situ*.

The bags were filled with 150 l unfiltered surface (0-0.25 m) water from the anchoring site. Nutrients were then added and thoroughly mixed with the water using a 30 cm $\varnothing$ Secchi disc. Number of bags, type and amount of nutrients added and length of the experiments varied for the four enrichment bioassays (Tab 1).

TAB 1. EXPERIMENTAL DESIGN.

EXPERIMENT/ DATE	SPIKE MG $\cdot$ M ⁻³		BAG			
A (270678- 040778)		P	NO ₃	PN1	PN2	
	PO ₄ -P	55	-	55	110	
	NO ₃ -N	-	850	850	1900	
B (030878- 110878)		NO ₃	NH ₄	PN0 ₃	PNH ₄	
	PO ₄ -P	-	-	55	55	
	NO ₃ -N	850	-	850	-	
	NH ₄ -N	-	960	-	960	
C (050878- 140878)		NO ₃	NH ₄	PN0 ₃	PNH ₄	
	PO ₄ -P	-	-	55	55	
	NO ₃ -N	850	-	850	-	
	NH ₄ -N	-	1280	-	1280	
D (230679- 010779)		P	NO ₃	NH ₄	PN0 ₃	PNH ₄
	PO ₄ -P	55	-	-	55	55
	NO ₃ -N	-	820	-	820	-
	NH ₄ -N	-	-	1000	-	1000

At the start of the experiments water (5 l) for analysis of particulate organic carbon (POC), chlorophyll α , ^{14}C uptake, nitrate, ammonium and phosphorus and for phytoplankton species identification was taken. Normally, 5 l water was removed daily from each bag after thorough mixing with the Secchi disc. Sampling was usually done at 8 am. Total phosphorus and total dissolved phosphorus were analyzed only in exp d and POC only in exp a. No ^{14}C uptake measurements were done in exp a.

POC was analyzed with a wet oxidation procedure (International Oceanography MSE Carbon Analyzer) using 5 replicate ampoules for each sample. Gelman glass fiber filters ($\emptyset$ 25 mm) with 1.0 μm retention capacity were used for filtration of particulate matter. Chlorophyll α was measured in material retained by a Whatman GF/F glass fiber filter (retention capacity 0.6 μm), which was dried in a dark, cool place and extracted with 90% acetone. The procedure followed the recommendations in Edler (1979) and used equations of Jeffrey and Humphrey (1975). For measurement of primary production subsamples were poured into 30 ml stoppered glass bottles together with 4 $\mu\text{Ci NaH}^{14}\text{CO}_3$. Bottles were incubated for two hours (9 - 11 a.m.) outside the bags approximately 30 cm below the surface. Filters were counted with a GM counter and cpm was corrected for dark uptake. Cpm was transformed to $\text{mg C}_{\text{fixed}} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ using measurements of pH, salinity and temperature and the formulae in Gargas (1975).

Nutrient analyzes were made on unfixed samples immediately upon return to the laboratory according to methods in Carlberg (1972). All analyzes were made at least in duplicate. GF/F glass fiber filters were used for fractionation of phosphorus. Particulate organic phosphorus (POP) was obtained through subtraction of total dissolved phosphorus

from total phosphorus (tot-P), and dissolved organic phosphorus (DOP) through subtraction of inorganic dissolved phosphorus ($\text{PO}_4\text{-P}$) from total dissolved phosphorus.

Water for qualitative and quantitative (Lugol fixation) phytoplankton analyses was taken in all experiments. This material is under analysis and will not be discussed in detail in this paper.

Normally, only one bag was used for each type of nutrient enrichment. In exp c some of the bags were duplicated, however. Replicate bags produced nearly identical concentrations of chlorophyll *a* (Fig 3), showing that reproducible results could be obtained within one experiment.

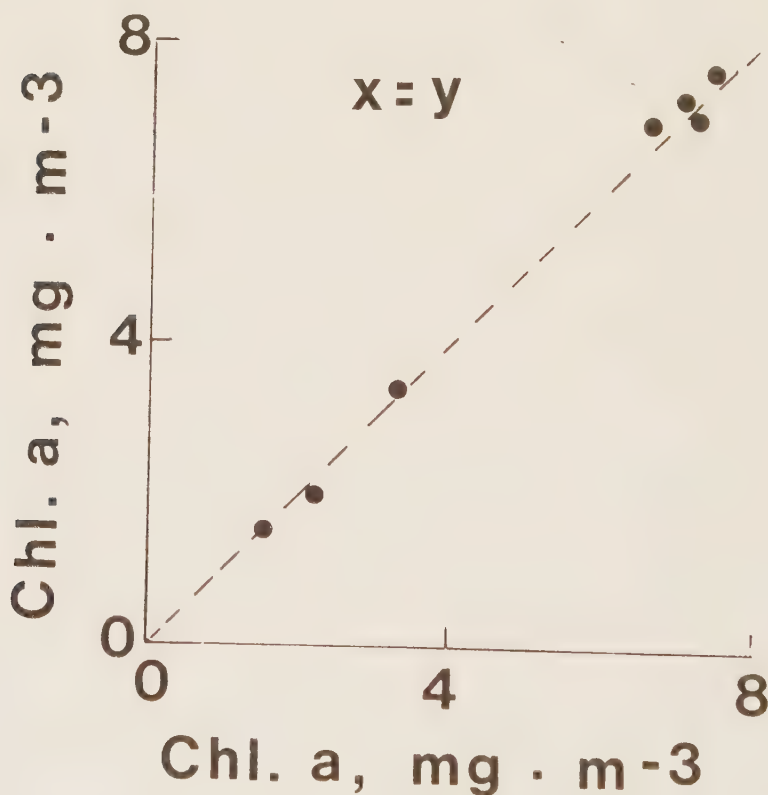


Fig 3. Chlorophyll *a* concentrations in two replicate bags with ammonia addition in exp c.

RESULTS

Weather conditions during the experiments

June 1978 was exceptionally warm and sunny with mean air temperatures substantially above the normal. However, July was significantly colder than normal in both 1978 and 1979, with few sunny days and much precipitation. August in both years was also colder than normal. Most of June 1979 was warm and sunny, but the weather was cold and unstable at the end of the month when experiments were carried out. Thus, as a whole, weather during the experiments was poor. Cold and windy weather might have prevented the formation of blooms of blue-green algae (*Nodularia spumigena*, *Anabaena* sp, *Aphanizomenon* sp), which are common in the South Baltic during late summer. Cold weather was reflected in low water temperatures, between 11 and 19°C for the 1978 experiments and between 15 and 19°C for 1979. Water temperatures in the area of the channel are subject to rapid fluctuations caused by changes in wind direction.

Chlorophyll *a*

Initial chlorophyll *a* concentrations in water used for the experiments were $<2 \text{ mg} \cdot \text{m}^{-3}$ (Tab 2). Generally the control bag (C) (without additions) showed the lowest chlorophyll *a* values among the different bags (cfr Fig 4). Phosphorus addition in exp a resulted in chlorophyll *a* concentrations similar to those in the control bag, while in exp d the control bag had higher chlorophyll *a* concentrations than the bag receiving phosphorus by the final days of the experiment (Fig 7). Nitrogen addition, in the form of ammonia or nitrate, stimulated chlorophyll *a* synthesis in all experiments, with maximal values between 4 and 9 $\text{mg} \cdot \text{m}^{-3}$. A simultaneous addition of both phosphorus and nitrogen further increased chlorophyll *a* concentrations compared to the nitrogen bags. In exp b the difference in chloro-

TAB 2. INITIAL AND MAXIMAL CHLOROPHYLL α CONCENTRATIONS IN THE BAGS OF EXPERIMENTS A TO D.

Exp	MAXIMAL CHLOROPHYLL α CONCENTRATIONS MG.M ⁻³ AND % OF CONTROL							
	INITIAL	C	P	NO ₃	NH ₄	PN ₃	PNH ₄	PN ₂
A	0.51	1.54	1.63	5.85	-	11.30	-	17.11
	%	100	106	380		734		1110
B	1.14	1.89	-	4.55	3.81	6.29	5.42	-
	%	100		241	202	333	287	-
C	1.51	5.51	-	8.55	7.70	27.88	22.75	-
	%	100		155	140	506	413	
D	0.68	3.28	2.16	6.19	3.90	26.82	51.18	-
	%	100	66	189	119	818	1560	

phyll α between the nitrogen and nitrogen plus phosphorus bags was not great, however (Fig 5). In exp c and d high chlorophyll α concentrations were produced after addition of nitrogen plus phosphorus (Fig 6 and 7). Chlorophyll α concentrations increased over initial values even in the control bags, especially in exp c and d. Maximal chlorophyll α values in the control bags were reached on different days for the four experiments, e.g. on the 5th day in exp a (Fig 4), and the 7th day in exp d (Fig 7). In exp b and c no peaks of chlorophyll α developed in the control bags (Fig 5 and 6).

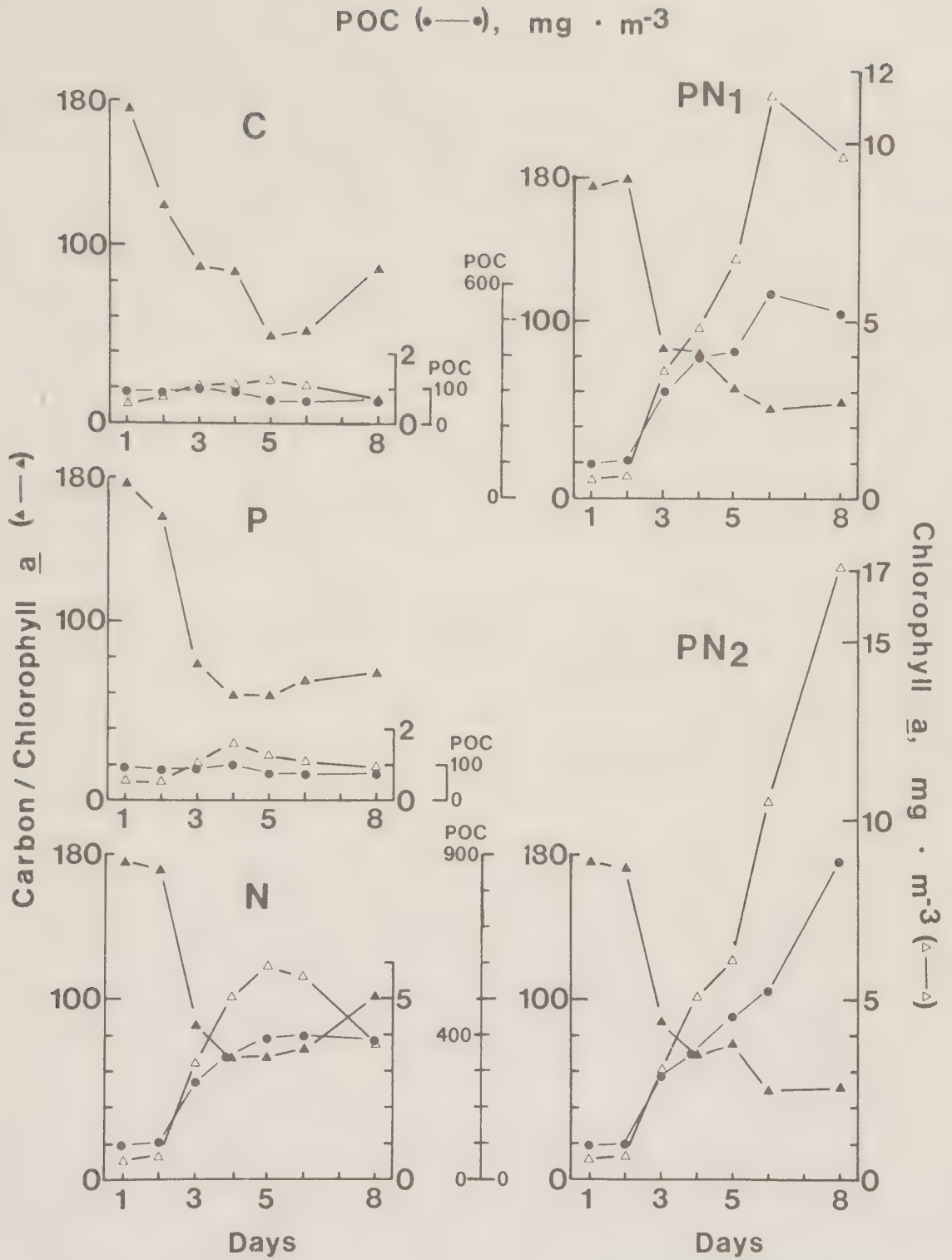


Fig 4. Chlorophyll $\bar{a}$, POC and POC/Chl $\bar{a}$ in the bags of exp a.

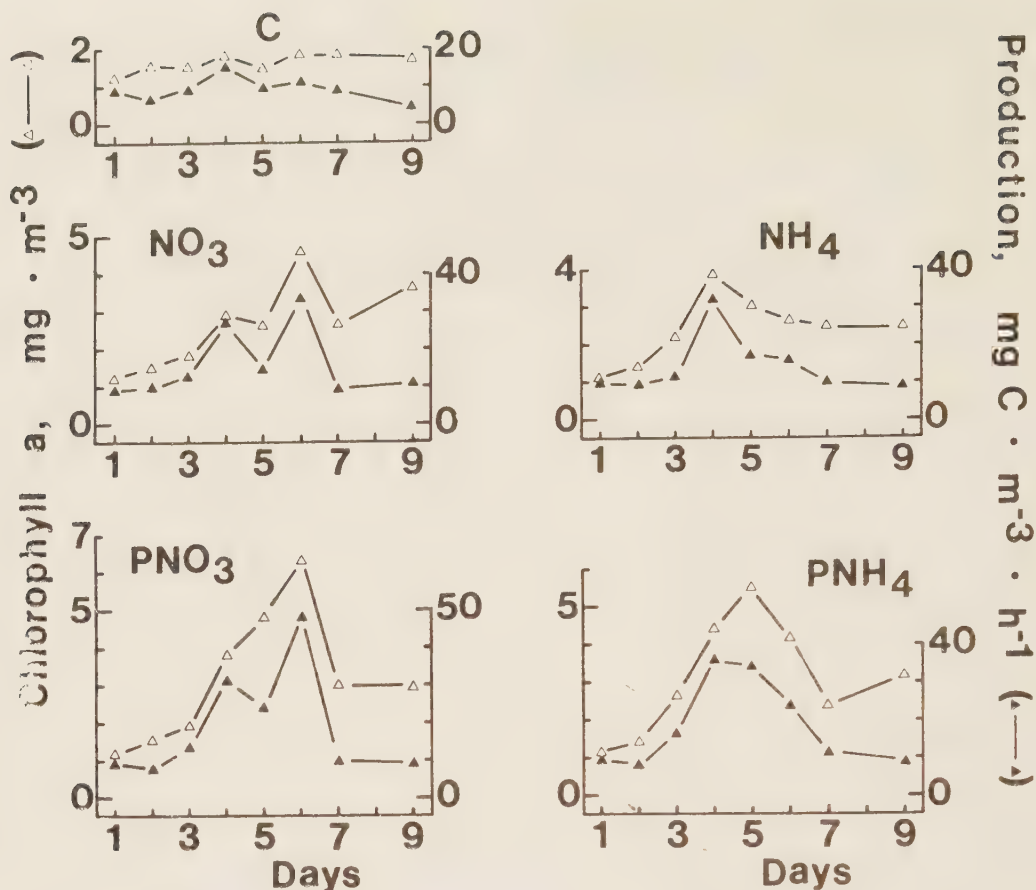


Fig 5. Chlorophyll *a* concentrations and primary production (¹⁴C fixation) in the bags of exp b.

Nitrate additions alone (exp b, c and d) always resulted in higher maximal chlorophyll *a* concentrations than ammonia additions, even though somewhat more nitrogen was added in the latter bags (Tab 1 and 2). When only nitrogen was added, no distinctive chlorophyll *a* peaks were formed in the bags of exp c and d.

In exp a two different levels of nitrate plus phosphate addition were tested. Biomass yield in terms of maximal amounts of chlorophyll *a* produced was not in proportion to nutrients added (Tab 2). Similar amounts of added nitrogen plus phosphorus in the different experiments gave very

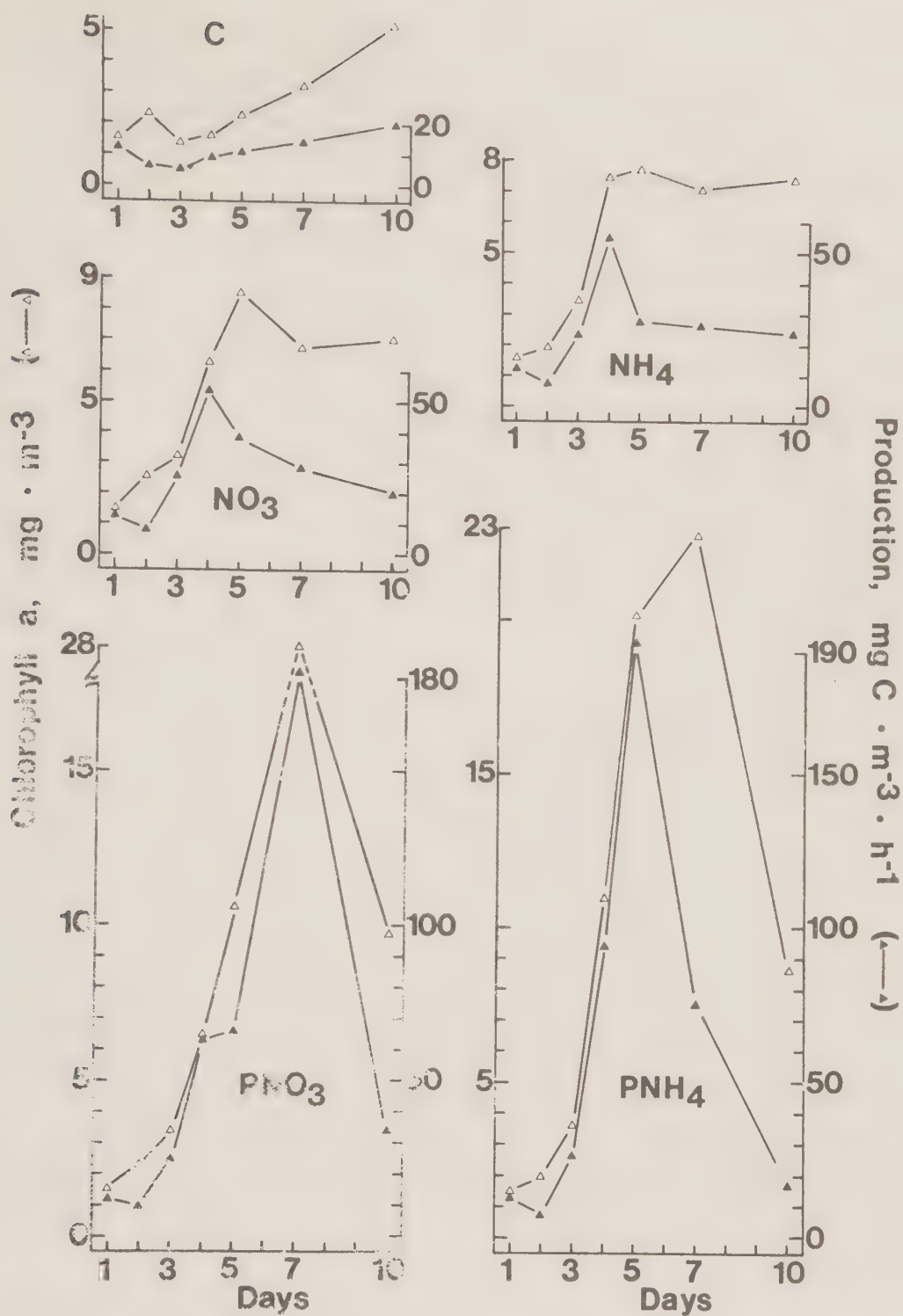


Fig 6. Chlorophyll *a* concentrations and primary production (¹⁴C fixation) in the bags of exp c.

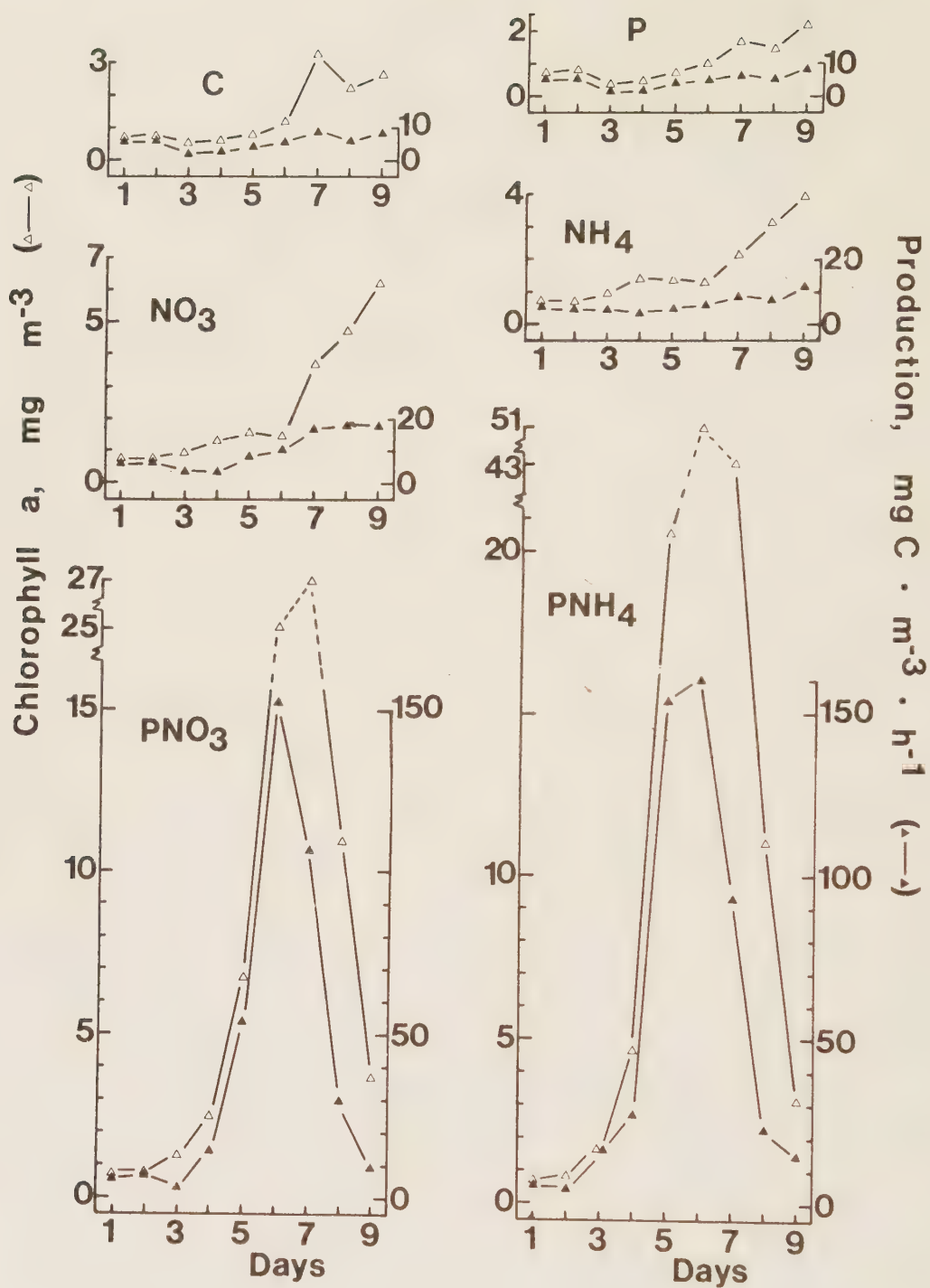


Fig 7. Chlorophyll *a* concentrations and primary production (¹⁴C fixation) in the bags of exp d.

different responses as chlorophyll *a*. Some other factor than nitrogen and phosphorus apparently limited phytoplankton growth in exp b, as only low chlorophyll *a* concentrations developed in all bags (Fig 5). In the other experiments a sharp increase in chlorophyll *a* was noted after nitrogen plus phosphorus addition, with peaks occurring on the 6th or 7th days. Thereafter, phytoplankton populations evidently collapsed in these bags. Chlorophyll *a* concentrations in the PNH_4 bags of exp c and d increased slightly earlier and at a higher rate than in corresponding PNO_3 bags (Fig 6 and 7). Chlorophyll *a* yield in the PNH_4 bag of exp d was almost twice that of the PNO_3 bag (Tab 2). In exp b and c there were only small differences between the PNO_3 and PNH_4 bags, however.

Particulate organic carbon

Concentrations of particulate organic carbon (POC) followed those of chlorophyll *a* for the bags in exp a (Fig 4). However, POC in control and phosphorus bags did not increase when chlorophyll *a* peaked. A least-squares fit between the two parameters gave the straight line: $\text{mg chl } a \cdot \text{m}^{-3} = 0.02 \text{ mg POC} \cdot \text{m}^{-3} - 1.29$ (Fig 8). Thus the overall weight ratio between POC and chlorophyll *a* was about 50:1. However, during the first two days of the experiment much higher ratios were obtained (Fig 4). Initial values were almost 180, while the quotient decreased to between 50 and 70 at the time of the chlorophyll *a* maxima.

Assimilation number

Carbon fixation by the phytoplankton communities of the bags followed the same general trends as chlorophyll *a* concentration, but with maxima occurring somewhat earlier (Fig 5-7). Assimilation numbers ($\text{mg C}_{\text{fixed}} \cdot \text{mg chl } a^{-1} \cdot \text{h}^{-1}$)

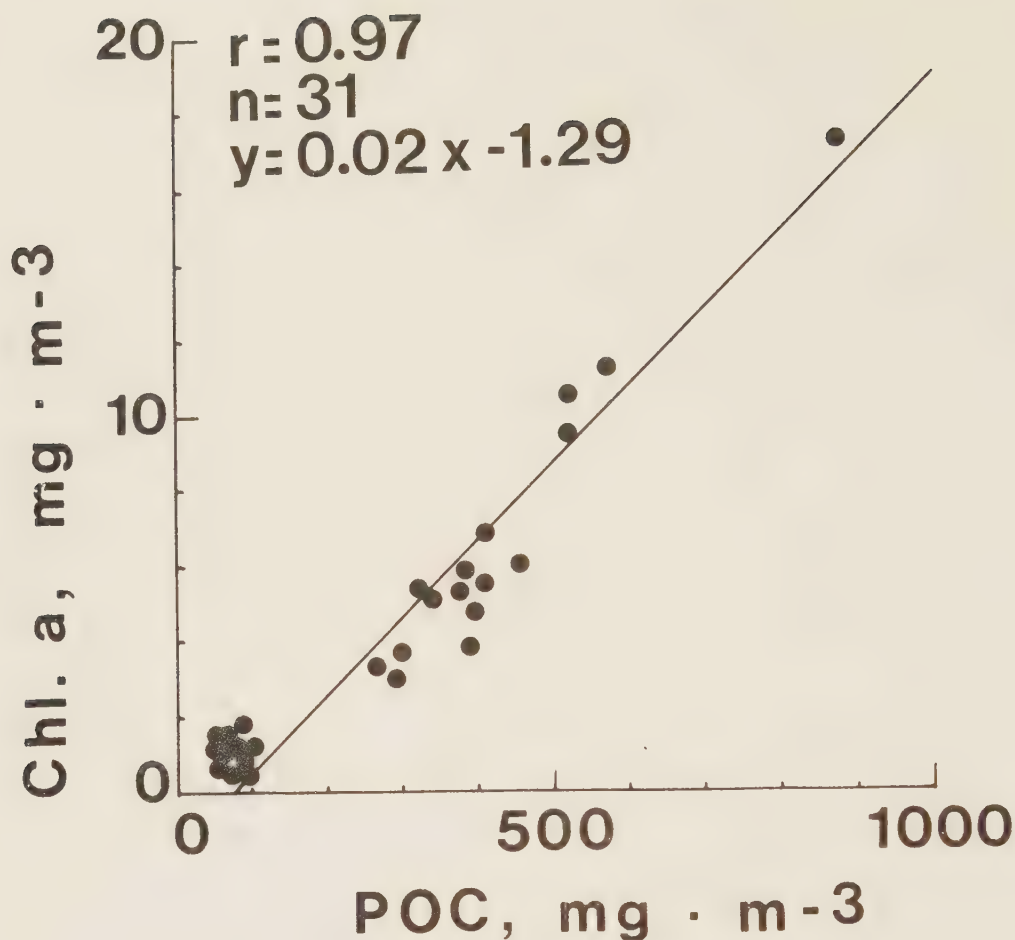


Fig 8. Correlation between POC and chlorophyll *a* in exp a.

were calculated for exp b, c and d (Fig 9). These ratios were not based on photosynthesis at light saturation but rather on the actual ¹⁴C fixation at 30 cm depth outside the bags. Initial values in the control bags were similar for the three experiments, between 8 and 9. An initial decrease was noted in most bags, followed by a peak in assimilation number and finally a substantial decrease. At the end of the experiments (day 9 or 10) assimilation numbers were around 3 in all bags. In exp b there was no clear difference in assimilation numbers between the enriched bags and the control bag (Fig 9). However, a difference

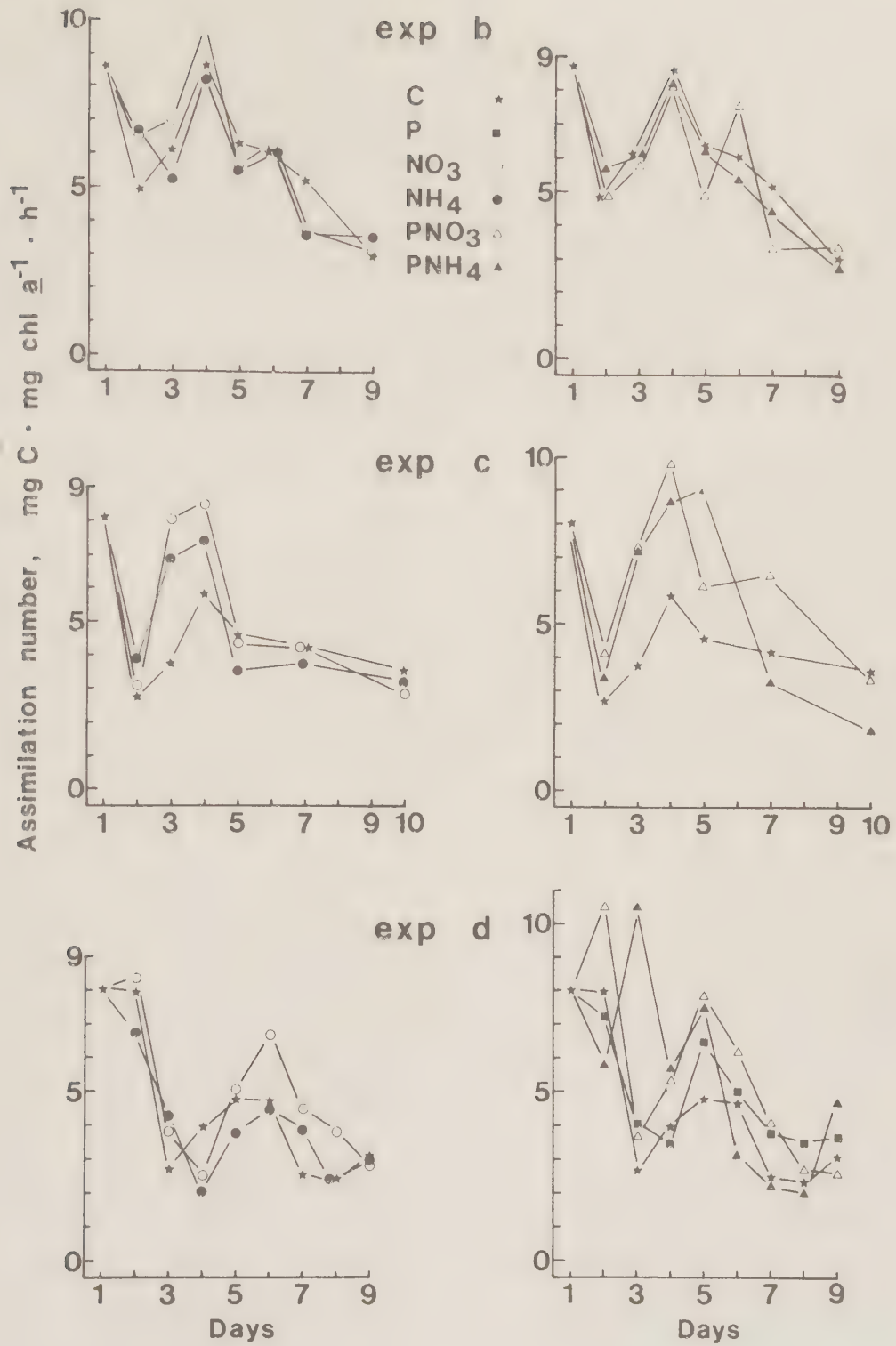


Fig 9. P_b quotients ($\text{mg C}_{\text{ass}} \cdot \text{mg chl } a^{-1} \cdot \text{h}^{-1}$) for the various bags in exp b, c and d.

was seen in exp c, especially for the 3rd and 4th days. Nitrate additions (alone or together with phosphate) gave slightly higher values than ammonia additions. Ammonia additions did not increase assimilation numbers over control bag values in exp d, while nitrate additions resulted in an increase (Fig 9). Even phosphate addition resulted in higher assimilation numbers compared to the control. The most marked effects were found after addition of both phosphate and nitrogen (nitrate or ammonia), however.

Phosphorus

Initial inorganic phosphorus concentrations in water used for the bioassay experiments varied between 6 and 12 mg $P \cdot m^{-3}$. In the control bags phosphorus concentrations decreased while chlorophyll *a* increased (see e.g. Fig 12). Addition of nitrate or ammonia resulted in more marked decreases in phosphate concentrations (Fig 10).

After addition of phosphate alone there was a steady decrease in phosphate concentration, a process which had not terminated by the end of the experiments (Fig 10 and 13). Mean net phosphate uptake in exp a and d with 55 mg $PO_4-P \cdot m^{-3}$ added initially was 6 and 5 mg $P \cdot m^{-3} \cdot d^{-1}$ for the entire experiments of 7 and 8 days, respectively.

Very efficient phosphate uptake occurred after simultaneous addition of phosphorus and nitrogen. The rate of phosphate uptake did not seem to be influenced by the amount of chlorophyll *a* produced. Phosphate disappeared as rapidly in exp b (Fig 11) with low chlorophyll *a* concentrations, as in exp c and d with high concentrations (Fig 12 and 13). Phosphate uptake showed a lag of a few days, whereafter phosphate concentrations dropped quickly (see e.g. Fig 10 and 11). Phosphate uptake rates were higher in the presence of ammonia than with nitrate (Fig 11 and 12). Maximal

Exp a

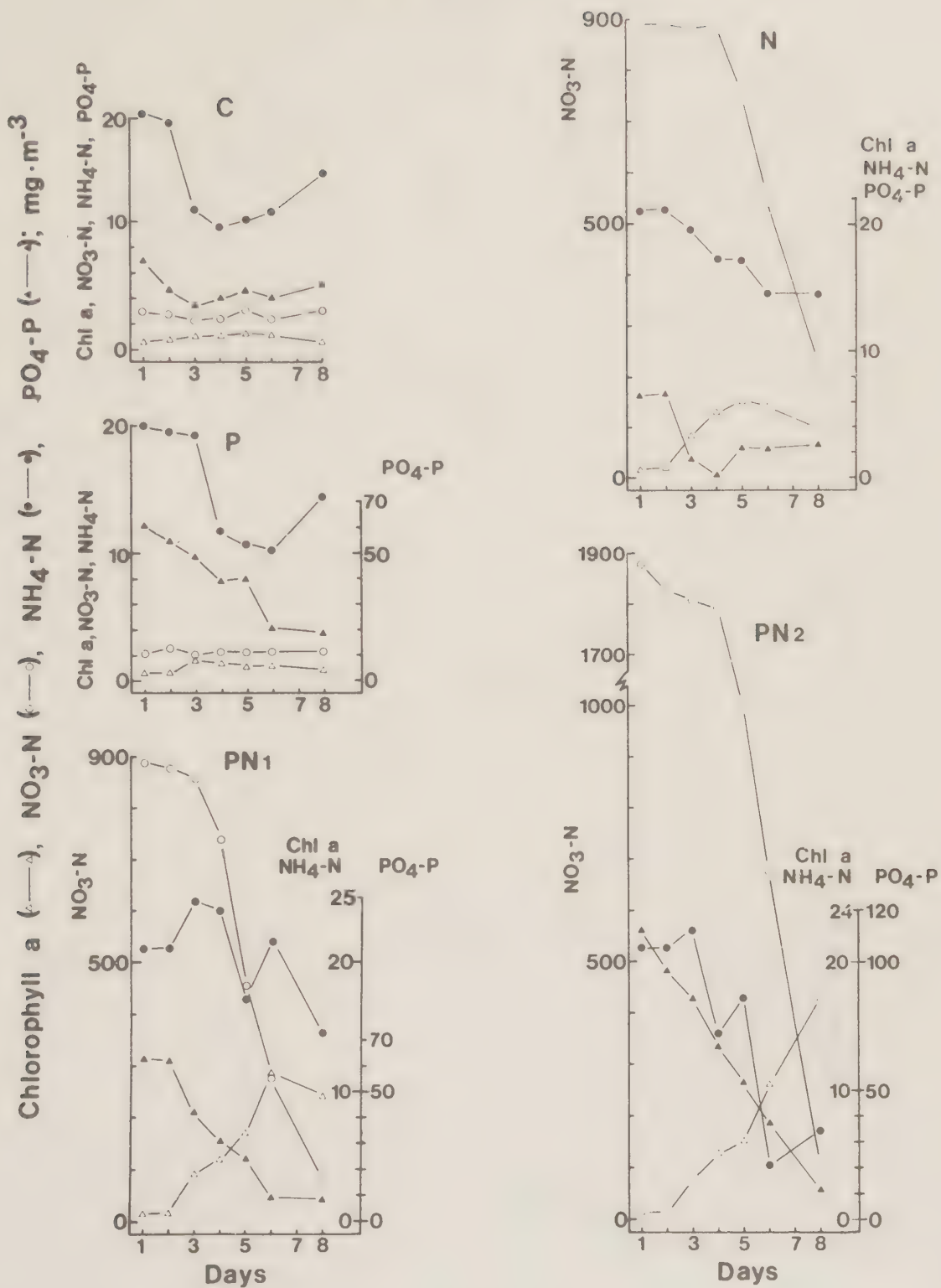
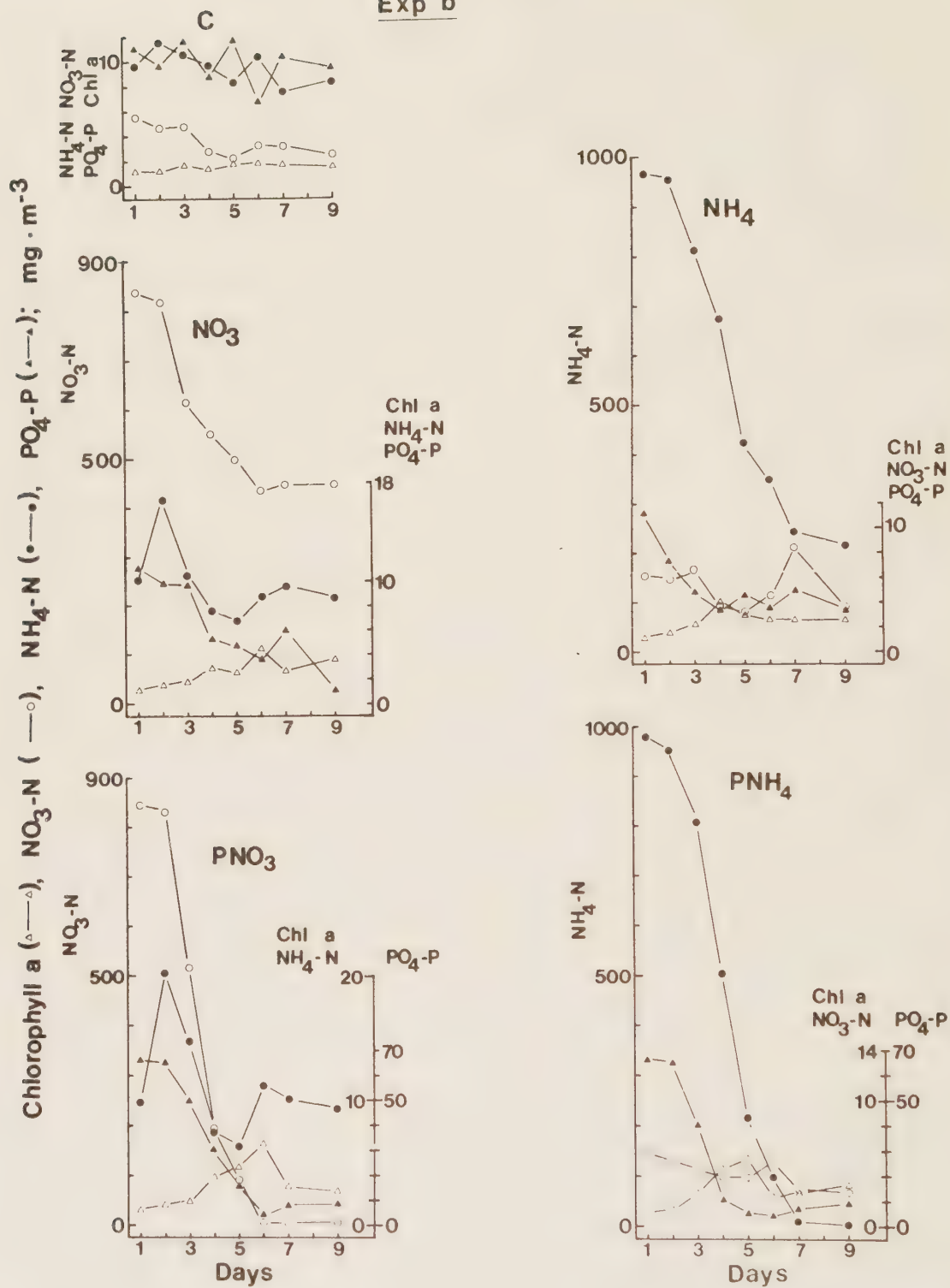


Fig 10. Chlorophyll *a* and nutrient concentrations in the bags of exp a.

Exp b



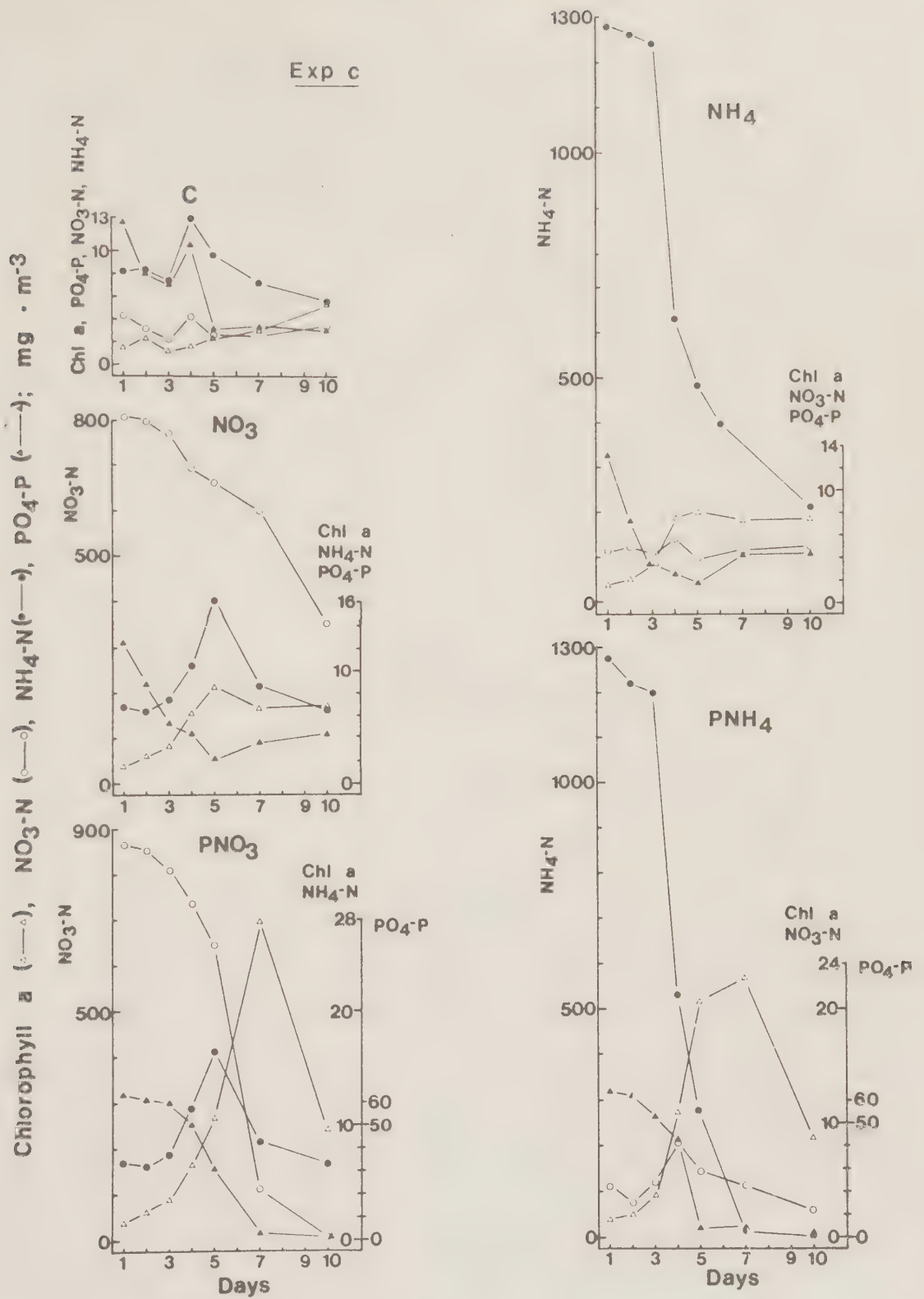


Fig 12. Chlorophyll *a* and nutrient concentrations in the bags of exp c.

Exp d

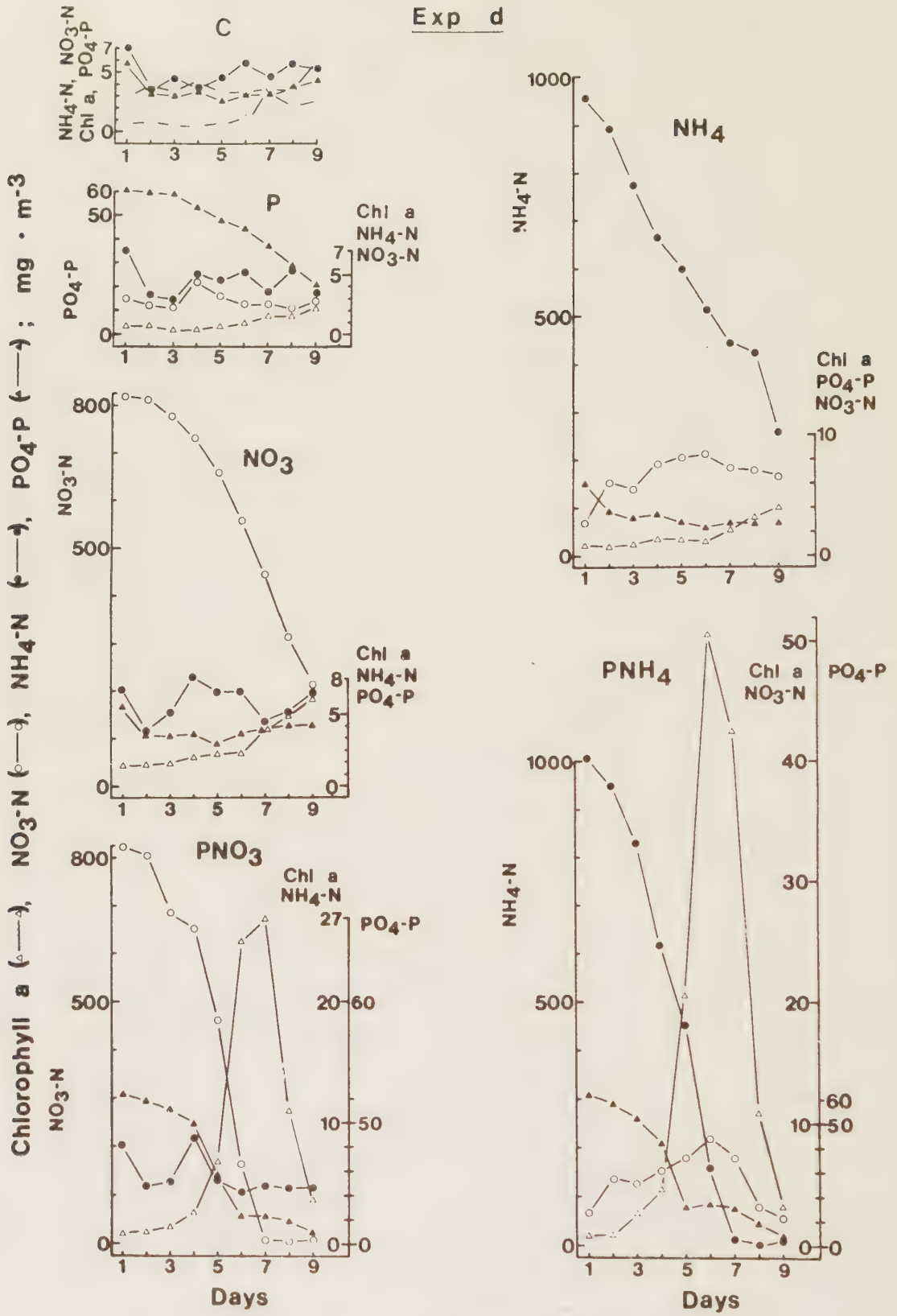


Fig 13. Chlorophyll *a* and nutrient concentrations in the bags of exp d.

uptake rates in the presence of added ammonia were 20 - 40 $\text{mg P} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. At the termination of the experiments, bags with additions of both phosphorus and nitrogen contained less than 10 mg inorganic dissolved $\text{P} \cdot \text{m}^{-3}$, similar to the amounts found in control bags.

In exp d different phosphorus fractions were analyzed (Fig 14). Only small changes occurred in the four phosphorus fractions in the control bag. DOP made up the bulk of 20 $\text{mg} \cdot \text{m}^{-3}$ tot-P. POP was a very small fraction of the phosphorus pool. Conditions were similar after addition of nitrate or ammonia, although POP increased somewhat.

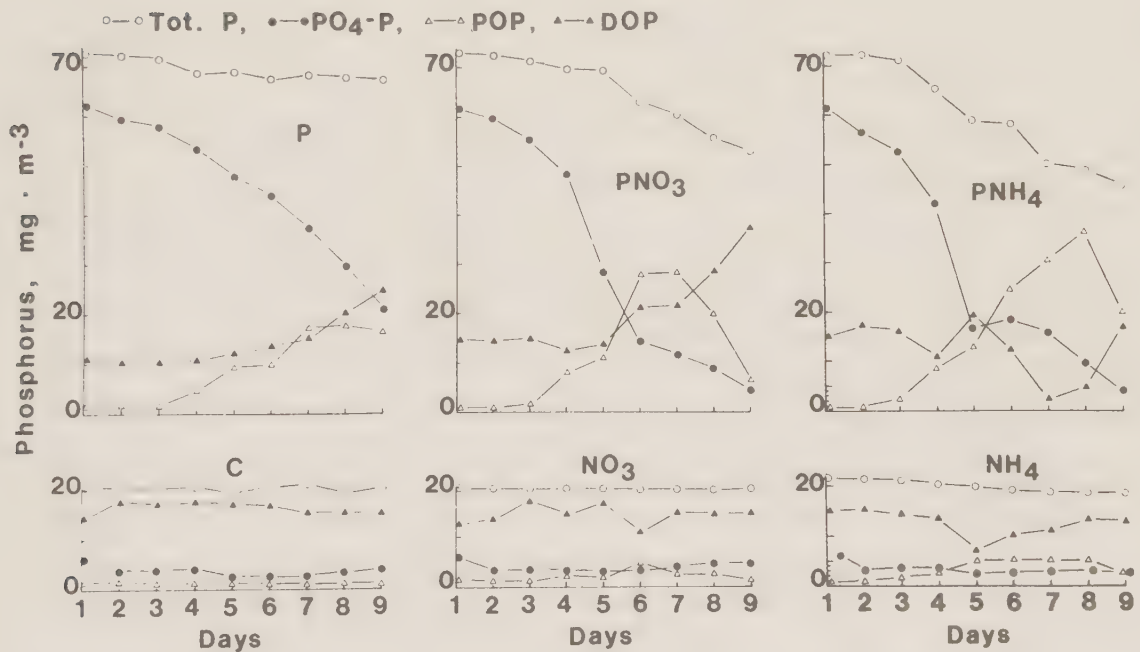


Fig 14. Phosphorus fractions in the bags of exp d.

When $\text{PO}_4\text{-P}$ was added alone to the water tot-P increased from $20 \text{ mg}\cdot\text{m}^{-3}$ to slightly above $70 \text{ mg}\cdot\text{m}^{-3}$ (addition $55 \text{ mg PO}_4\text{-P}\cdot\text{m}^{-3}$). During the course of the experiment (8 days) only $5 \text{ mg P}\cdot\text{m}^{-3}$ disappeared. Although tot-P remained constant, the other phosphorus fractions changed greatly. A decrease in $\text{PO}_4\text{-P}$ was followed by an increase in DOP, which had doubled by the end of the experiment. POP increased even more dramatically, from less than 1 to more than $15 \text{ mg}\cdot\text{m}^{-3}$.

With addition of nitrate or ammonia in combination with phosphate, relations between the phosphorus fractions were different. Tot-P decreased with about $20 \text{ mg}\cdot\text{m}^{-3}$ in the PNO_3 bag and about $25 \text{ mg}\cdot\text{m}^{-3}$ in the PNH_4 bag (Fig 14). The disappearance of phosphorus is probably explained by growth of periphyton on the walls. Such growth was visible, but because of the short duration of the experiments, the periphytic community was probably still in an early developmental stage with low biomass. Sedimentation is a less likely explanation for the disappearance of phosphorus as the water in the bags was vigorously mixed before sampling. POP reached peaks of 30 and $40 \text{ mg P}\cdot\text{m}^{-3}$ in the two bags, but decreased at the end of the experiment. DOP increased steadily in the PNO_3 bag but fluctuated in the PNH_4 bag. The most rapid uptake of $\text{PO}_4\text{-P}$ occurred between the 4th and 5th days.

Inorganic nitrogen

Initial concentrations of nitrate in water used for the bioassay experiments varied between 3 and $5 \text{ mg N}\cdot\text{m}^{-3}$. Ammonia had a somewhat higher range, up to $20 \text{ mg NH}_4\text{-N}\cdot\text{m}^{-3}$. In the control bags no dramatic changes occurred in nitrate and ammonia concentrations. The same was true for the phosphorus bags (Fig 10 and 13).

Without a simultaneous addition of phosphorus, inorganic nitrogen did not drop below $200 \text{ mg N} \cdot \text{m}^{-3}$ after addition. Ammonia decreased at a higher rate than nitrate in exp b and c (Fig 11 and 12), but not in exp d (Fig 13).

Nitrogen uptake was very efficient after simultaneous addition of both inorganic nitrogen and phosphate, with final inorganic nitrogen concentrations close to zero (see e. g. Fig 13). As was the case for phosphate, the rate of nitrogen consumption and the total amount of nitrogen consumed showed no relation to the amount of chlorophyll *a* produced (cf. Fig 11 and 12).

DISCUSSION

There are obvious advantages in conducting enrichment bioassays in bottles or test tubes in the laboratory: easy handling, the possibility of using large numbers of replicates and different additions, and standardized environmental conditions (light, temperature and mixing). In addition laboratory tests usually do not suffer from the influence of storms, boats, or curious human beings and animals. On the other hand laboratory conditions are different from the natural environment, e.g. with respect to temperature, light and turbulence. Because of high surface to volume ratios in bottles and test tubes, wall effects such as adsorption and bacterial growth can occur.

The above problems are not felt to exist for enclosures anchored *in situ*. As a consequence, very large plastic spheres and cylinders have been developed for phytoplankton studies *in situ* (McAllister et al. 1961, Brockmann et al. 1977). However, every delimitation of an ecosystem is unnatural and prevents interactions with other parts of

the ecosystem. Interactions with the bottom and higher trophic levels, e.g. fish, are usually excluded. The presence or absence of zooplankton, bottom fauna and benthic- or planktivorous fish can greatly modify structure and function of phytoplankton communities (Andersson et al. 1978). In addition, the water under study is stationary instead of being in constant movement, both vertically and horizontally.

Enclosure of water in the plastic bags induced an immediate response in the phytoplankton community. In the control bags chlorophyll *a* concentrations increased while inorganic nitrogen and phosphorus either decreased or remained at initial concentrations (cf. e.g. Fig 12). Chlorophyll *a* synthesis in the control bags was probably not accompanied by an actual increase in phytoplankton biomass. In exp a POC actually decreased from the 1st to the 5th day in the control bag while chlorophyll *a* increased from 0.5 to 1.5 mg·m⁻³ (Fig 4, Tab 2). In exp d an even stronger increase in chlorophyll *a* was noted in the control bag, from 0.7 to 3.3 mg·m⁻³. Here, inorganic nitrogen (ammonia plus nitrate) remained around 9 mg N·m⁻³ (Fig 13), while PO₄-P decreased initially and then slowly increased.

Thus it was difficult to couple the increase in chlorophyll *a* concentrations in control bags to nutrient uptake. Concentrations of phosphate, ammonia and nitrate in the control bags were so low, however, that slight analytical errors could greatly influence these results. Another factor which might have influenced chlorophyll *a* concentrations in the bags is light climate. Light conditions for a phytoplankton cell in a bag where nitrogen and phosphorus were not added simultaneously were probably better than for a cell in the free water which would spend more time in deeper and thus darker layers. On the other hand, cells in the bags would be more liable to

photoinhibition, as mean transparency in the south part of the Öresund around 7 m (Edler 1977). Improved light climate could also lead to a decrease in chlorophyll *a* concentrations in the cells (sun adaption). Substantial chlorophyll *a* increase has been noted in bottle experiments with Öresund water without additions (Granéli 1980). In this case, however, samples were taken during periods with higher inorganic nutrient concentrations than in the present experiments, and the bottles were incubated under continuous illumination.

Finally, species successions took place in the phytoplankton communities of the bags. In the control bag of exp d there was a change from a dominance of *Oocystis* sp. and *Leptocylindricus danicus*, over *Aphanothece* sp. to *Rhodomonas* spp. and *Cylindrotheca closterium*. This could change chlorophyll *a* concentrations even if the phytoplankton biomass did not increase. Rapid changes in phytoplankton communities seem to be the rule in enrichment experiments (cf. Granéli op. cit.).

Chlorophyll *a* development in replicate bags was almost identical (Fig 3). Exp b and c, which were started only two days apart, gave quantitatively very different results, however. This difference can hardly be explained by changes in weather conditions or initial nutrient and chlorophyll *a* concentrations. A possible cause is plankton patchiness, which would allow selection of different plankton species in the two experiments. Exchange of water in the Falsterbo Channel is extremely rapid (Svensson 1978). The current can reverse direction in response to wind and air pressure, which means that water in the channel is sometimes derived from the Baltic proper, sometimes from the Öresund.

A typical phenomenon in experiments with an initial, large addition of nutrients is the rapid buildup of a phytoplankton population followed by a sudden collapse. This development is especially evident in exp c and d for the PNO_3 and PNH_4 bags. Collapse occurred simultaneously with depletion of inorganic nitrogen and phosphorus after about one week. Comparable situations do exist in the natural environment, e.g. during the spring outburst of diatoms in the Öresund.

A sudden, large addition of nutrients to a nutrient-poor water does not resemble long-term nutrient enrichment through upwelling or cultural eutrophication, however, as the time for ecosystem adaption is much too short. Recycling of nutrients does not seem to occur in the bags, for example. The results of the present experiments should therefore be considered qualitative with respect to nutrient limitation. In the Öresund, the actual ecosystem response to a permanent increase in P and N of the magnitude used here would probably be quantitatively different from the response of the plankton communities in the bags.

It is generally accepted that nitrogen limitation is common among oceanic and coastal phytoplankton communities (although examples of phosphorus limitation do exist, e.g. Berland et al. (1978)). The Baltic is a brackish extension of the North Sea, with surface salinities below 10 ‰. Influence from river water is thus substantial, and many freshwater phytoplankton genera are found, e.g. *Aphanizomenon*, *Anabaena*, *Selenastrum*, *Oocystis* and *Dinobryon*. Because of the freshwater influence and locally strong pollution, one cannot *a priori* assume that nutrient limitation conditions in the Baltic are identical to those in "pure" sea water.

The Öresund receives vast quantities of domestic and industrial sewage (Damgaard 1980), but because of the enormous throughflow, nutrient concentrations are still relatively low (Dahl-Madsen 1980). Conditions in the Öresund with respect to nutrient limitation seem simple. As has been shown in earlier investigations (Granéli 1978 and 1980) phosphorus additions rarely stimulate phytoplankton growth (only in late summer when heterocyst-carrying cyanophyceans appear in "blooms"). Nitrogen enrichment practically always enhances chlorophyll *a* synthesis and ^{14}C fixation, although the change in plasma volume may not be in proportion to the increase in chlorophyll *a* (Granéli in press).

The present *in situ* experiments showed stimulation of phytoplankton growth by nitrogen enrichment, but no positive effects were found after phosphorus addition. All the experiments were made in June or August. Because of unfavourable weather conditions, no heavy development of heterocyst-carrying blue-green algae occurred in August 1978 or 1979. These algae can fix molecular nitrogen in late summer when inorganic nitrogen concentrations are low, and this process is stimulated by phosphorus addition (Granéli and Sundström in prep). Phosphorus addition to Öresund water in laboratory tests has resulted in increased chlorophyll *a* concentrations on a few occasions. This has happened in August when *Nodularia spumigena* has been present in large numbers (Granéli 1980).

In earlier laboratory tests with natural phytoplankton populations from the Öresund, nitrogen stimulation was - with the rare exception noted above - evident throughout the year (Granéli 1978 and 1980). Therefore it is probable that results from the *in situ* experiments in the Falsterbo Channel are representative for all seasons. Laboratory

tests were made with water from the Öresund sampled between Malmö and Kullen (where the Öresund merges with the Kattegat). In the central part of the Öresund (between Saltholm and Ven) influence from sewage is evident, and concentrations of phosphorus and nitrogen are increased about 10 and 100 $\text{mg}\cdot\text{m}^{-3}$, respectively (Dahl-Madsen 1980, von Wachenfeldt 1980).

Water in the Falsterbo Channel is not directly influenced by sewage and is probably representative for Baltic water flowing into the Öresund. It can thus be concluded that eutrophication of the Öresund has not affected the most limiting nutrient for phytoplankton growth and that nitrogen should be the most limiting nutrient even in the South Baltic.

Unfortunately, there are no investigations (through enrichment experiments) of nutrient limitation of phytoplankton production in the open Baltic, Gulf of Bothnia or Bothnian Bay. However, some work has dealt with the archipelagoes of Stockholm (Melin and Lindahl 1973, Norin 1975) and Helsinki (Rinne and Tarkiainen 1978). These studies are from strongly-polluted areas where conditions are not comparable to the South Baltic or the Öresund. In the Stockholm and Helsinki archipelagoes no single nutrient limits phytoplankton during the entire year. Different nutrients or combinations of nutrients have been shown to stimulate phytoplankton growth depending on season and distance from the most polluted area (Norin op. cit., Rinne and Tarkiainen op. cit.). Phosphorus seems to play an important role through stimulation of nitrogen-fixing blue-green algae (Lindahl et al. 1980). In the unpolluted Askö archipelago south of Stockholm, Horstmann (1975) showed the importance of phosphorus for heterocyst production in enrichment experiments *in situ* during the summer. For the Swedish west coast no

enrichment experiments seem to have been made, and eutrophication has mainly been discussed in connexion with phosphorus (Söderström 1979).

Response to nutrient enrichment has been discussed here in terms of yield as chlorophyll *a*. In a pelagic community with heavy phytoplankton losses due to grazing and sinking, increased concentrations of inorganic nutrients might not lead to a biomass increase. Instead, growth rate or production per chlorophyll *a* unit may increase as a result of nutrient enrichment. The influence of nitrogen deficiency on $P_{\max}$ (carbon assimilation per unit chlorophyll *a* at light saturation, $\text{mg C} \cdot \text{mg chl } a^{-1} \cdot \text{h}^{-1}$) has been discussed by Thomas (1970). He found a mean $P_{\max}$ for nitrogen-poor water of 3.15, while the mean value was 4.95 in nitrogen-rich water (from the tropical Pacific Ocean). Low $P_{\max}$ values due to silica depletion have been found for an eutrophic lake by Coveney (1980).

In my study the relationship between light and photosynthesis was not studied. Thus, it is not clear if the calculated assimilation numbers represent $P_{\max}$. It is probable that light saturation occurred, as samples were incubated from 9 to 11 a.m. at approximately 30 cm depth under good light conditions. Light inhibition might have occurred, however, since water in the channel is quite clear. The absolute values of assimilation numbers should therefore be interpreted with caution. It is possible to compare values from the different bags within each experimental series, however.

In exp b enrichment with both nitrogen and phosphorus did not increase phytoplankton growth substantially (Fig 5). Assimilation numbers were high both in control and in the enriched bags at the beginning of the experi-

ment (Fig 9). Judging from the assimilation numbers, the phytoplankton were not nutrient limited. Concentrations of $\text{PO}_4\text{-P}$, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were on the order of 10, 5 and 10 $\text{mg}\cdot\text{m}^{-3}$ during the initial days of the experiment. Although these values are low, they are (with respect to nitrogen) still in the range of measured half-saturation constants for nitrogen uptake. McIsaac and Dugdale (1969) suggested that for eutrophic tropical seas, K_s (half-saturation constant) for nitrate uptake is $< 1.0 \text{ } \mu\text{g-at}\cdot\text{l}^{-1}$ (14 $\text{mg NO}_3\text{-N}\cdot\text{m}^{-3}$). Caperon and Meyer (1972) measured K_s values for nitrogen uptake by several species of marine phytoplankton grown with ammonia or nitrate as the limiting nitrogen source in a chemostat. Their mean K_s values were 0.32 and 0.24 $\mu\text{g-at}\cdot\text{l}^{-1}$ (4.5 and 3.4 $\text{mg N}\cdot\text{m}^{-3}$) for nitrate and ammonia, respectively. As half-saturation constants for growth are lower than corresponding constants for nitrogen uptake (Caperon and Meyer op. cit.), nitrogen should not have limited growth in the bag experiments. On the other hand marine or fresh water phytoplankton under salinity stress might not be as efficient in terms of nitrogen uptake as the above examples indicate (cf. Edler 1977).

In exp c nitrogen enrichment (ammonia or nitrate) increased assimilation numbers over the control values, with a further stimulation from a simultaneous phosphate addition. Except for the initial value, assimilation numbers of the control bag were low. The initial decrease, which was also noted in the other experiments, was caused by reduced rates of ^{14}C fixation during the second or third day of the experiment (see e.g. Fig 6 and 7). This decrease in primary productivity was not accompanied by a lowering of the chlorophyll a concentrations. Background nutrient concentrations in exp b and c were almost identical, so the lack of response in assimilation numbers after nutrient enrichment in the former case cannot be explained by different nitrogen or phosphorus

oncentrations. In the last experiment, d, which was run at the end of June (b and c in the beginning of August), nutrient enrichment also had an effect on assimilation numbers. Background nutrient concentrations were lower in this experiment, roughly $5 \text{ mg} \cdot \text{m}^{-3}$ for $\text{PO}_4\text{-P}$, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$. Except for the initial value, assimilation numbers in the control bag were low which might indicate nitrogen deficiency according to the findings of Thomas (op.cit.).

Harrison and Platt (1980) investigated variation in assimilation numbers for phytoplankton from a coastal (enriched) bay in Nova Scotia, Canada. Through multiple regression analysis they concluded that temperature accounted for 40 % of the observed variation. Phytoplankton species composition also contributed significantly to the variation in assimilation numbers, while there was little influence of nitrate concentrations. In enrichment experiments with natural North Pacific phytoplankton communities, Glooschenko and Curl (1971) found little enhancement in assimilation numbers in contrast to some of the results presented here. On the other hand, Granéli (1981) noted a marked increase in assimilation numbers in *in situ* experiments in the Fästerbo Channel following enrichment, especially with primary sewage.

As a whole, it seems that from a growth rate point of view phytoplankton populations in the bags were not severely nitrogen (or phosphorus) limited. On the other hand phosphorus must have been in excess in the water in relation to nitrogen. N/P quotients in the bag water before enrichment were 3.2, 1.4, 1.0 and 1.7 (inorganic fractions, weight ratios) for exp a, b, c and d. These low N/P ratios clearly indicate relative nitrogen deficiency, as measured quotients in phytoplankton from the Baltic are about 6 (Sen Gupta and Koroleff 1973) and even higher in oceanic water (Haug et al. 1973). Thus it is not totally clear that nit-

rogen concentrations in the Öresund are low enough to limit phytoplankton growth rate, but it seems clear that nitrogen is limiting the potential biomass yield in relation to phosphate.

Nitrate was originally considered the most important nitrogen source for marine phytoplankton. Modern investigations have emphasized the importance of ammonia (Eppley et al. 1979) and urea (McCarthy 1972) as additional nitrogen sources. In Öresund surface water average ammonia concentrations are about one third of average nitrate concentrations (20 and 60 mg N·m⁻³, von Wachenfeldt 1980) but in the present investigation background ammonia concentrations were generally higher than nitrate concentrations. Paasche (1971) showed that the marine flagellate *Dunaliella tertiolecta* grew faster with NH₄ than with NO₃. A similar effect could not be detected for the mixed phytoplankton populations in the bags, in accordance with findings of Eppley et al. (1971).

In exp b and c the yield in terms of chlorophyll *a* was similar for bags enriched with ammonia and nitrate when phosphate was added simultaneously, while in exp d the PNH₄ bag produced almost twice the chlorophyll *a* concentration in the PNO₃ bag. This difference can hardly be explained by the somewhat higher addition of ammonia in comparison to nitrate (1000 and 820 mg N·m⁻³, respectively). Conover (1975) did not find significant differences in growth rate or cell yield for cultures of *Thalassiosira fluviatilis* enriched with nitrate, ammonia or urea. There is no reason to believe that such differences should exist even if nitrate requires more energy than ammonia for incorporation in the cell.

In laboratory investigations it has been shown that ammonia concentrations above about 1 µg-at·l⁻¹ (14 mg N·m⁻³)

suppress nitrate uptake (Conway 1977). In field investigations using ^{15}N -tracer techniques, McCarthy et al. (1977) found that ammonia and urea are preferentially used compared to nitrate and nitrite by estuarine phytoplankton over a wide range of salinities and temperatures. Background concentrations of nitrate and ammonia were so low in the bags that any depression of nitrate uptake by ammonia would be difficult to detect. However, in similar experiments with sewage enrichment it was found that nitrate was not utilized until ammonia had reached very low concentrations (Granéli 1981).

Although phosphate additions alone did not give higher production, chlorophyll a or POC concentrations than in control bags, added phosphate rapidly disappeared. In the P bag phosphate did not leave the water to any great extent through adsorption to the walls or sedimentation, but was mainly transformed to particulate and dissolved organic forms. The question is, what type of organisms mediated this transformation? Chlorophyll a increased substantially in the P bag of exp d (from 0.7 to $2.2 \text{ mg} \cdot \text{m}^{-3}$), but not in proportion to the POP increase (from less than 1 to $17 \text{ mg} \cdot \text{m}^{-3}$).

Fractionation of chlorophyll a and POP was done with Whatman GF/F glass fiber filters, which have a mean retention capacity corresponding to $0.6 \mu\text{m}$. Although a complete separation of phytoplankton and heterotrophic microorganisms (bacteria, fungi) cannot be made through filtration, it has been shown that most heterotrophic activity is associated with the $< 1 \mu\text{m}$ fraction (Harrison et al. 1977). However, Salonen (1979) has shown that GF/F filters retain a high percentage of bacteria in lake water. Therefore, POP in my experiments does not include only phytoplankton, but also some bacteria (in addition to detrital particles).

In the bag with added phosphate the POP/chl α quotient (w/w) increased rapidly from less than one to over 10 (Fig 15), while in the control bag the mean quotient was 0.4. Eppley et al. (1971) found a POP/chl α quotient for unenriched natural phytoplankton (mainly diatoms) of 1.1, whereas nitrate- and ammonia-enriched shipboard (mixed species) cultures had mean quotients (stationary growth phase) of 2.1 and 1.6, respectively. McAllister et al. (1961) reported values from 0.8 to 1.4 for natural phytoplankton growing in a large-volume plastic sphere under "high nutrient" conditions. Values found in the present investigation for the PNO_3 and PNH_4 bags were in the same range when phytoplankton growth had reached the end of the exponential phase (cf. Fig 7 and 15). For *Skeletonema costatum* Harrison et al. (1977) found a relatively stable POP/chl α quotient (2.8) for low growth rates in a chemostat when phosphorus was not limiting growth. Kredel (1979) working with mixed phytoplankton populations in chemostats under natural light and temperature conditions found POP/chl α quotients between 0.4 and 16.2, with a mean of 3.2 (n=32). These algal populations grew under non-limiting phosphate concentrations. My values for the control bag were low compared to the above investigations. This might indicate phosphorus deficiency in the phytoplankton, and indeed phosphate concentrations in the water were low. However, when nitrogen was added the quotients increased substantially (Fig 15), showing that there was an adequate supply of phosphorus in relation to background nitrogen concentrations. Thus the initial, low POP/chl α quotients did not indicate phosphorus deficiency in the phytoplankton.

It can be assumed that the high POP/chl α quotients in the phosphate-enriched bag indicate luxury phosphate uptake by phytoplankton. Because of lack of nitrogen the incorporated phosphorus could not be utilized in synthesis of biomass. Part of the POP was transformed to DOP, especially

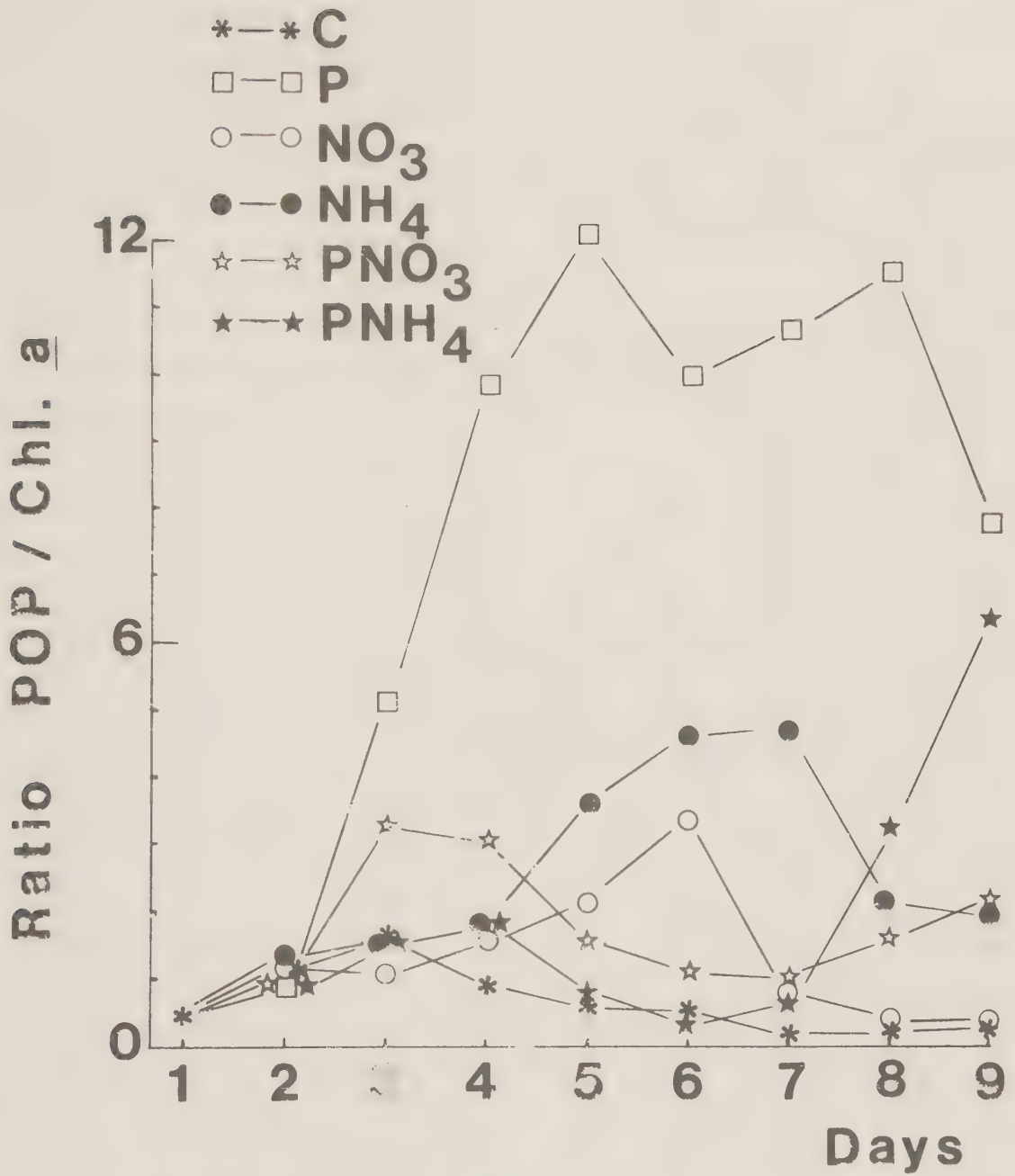


Fig 15. POP/chl *a* quotients in the bags of exp d.

in the PNO_3 and PNH_4 bags after decline of the phytoplankton populations. As a consequence of transformations by planktonic organisms, the dissolved inorganic phosphorus is rapidly removed from the water and appears as POP and DOP. In this form it is probably not directly available to phytoplankton. In a real ecosystem some of the organically-bound phosphorus would become available to the phytoplankton through mineralization and through passage through grazing food chains.

In my enrichment experiments chlorophyll a was used as a measure of phytoplankton biomass. Since chlorophyll a can be synthesized independently of cell divisions, and since chlorophyll a concentration in phytoplankton cells is a function of light and nutrient status (Beale and Appleman 1971, Laws and Bannister 1980), it was desirable to check chlorophyll a values against another measure of biomass. In exp a POC was analyzed and compared to chlorophyll a . The comparison is complicated by the fact that POC measured on total particulate matter also includes detrital material. Detrital POC can be estimated through regression analysis of POC and chlorophyll. Extrapolation gives detrital POC as the y-intercept (Steele and Baird 1962a, Tett et al 1975), while the slope of the regression line gives the C/chl a ratio. The data from exp a resulted in an intercept of $64 \text{ mg POC} \cdot \text{m}^{-3}$ and a C/chl a quotient of 50 (Fig 8). The most important underlying assumption in this calculation is that detrital POC does not vary systematically with chlorophyll. This assumption is generally not warranted (Banse 1977), and is probably not correct in the present case either, as detrital POC should increase when phytoplankton populations collapse in the bags.

C/chl a ratios for the particulate matter (including phytoplankton, bacteria, fungi, zooplankton and detrital material) in the bags ranged from about 50 to 180. The higher

figures were recorded on the first two days of the experiment. At the time of the chlorophyll *a* peaks, values ranged from 50 to 70. In a similar experiment C/chl *a* was calculated on the basis of algal plasma volume (Granéli in press). Based on regression analysis, quotients for low chlorophyll *a* values ($<6 \text{ mg} \cdot \text{m}^{-3}$) were roughly 70 and for higher chlorophyll *a* values ($>10 \text{ mg} \cdot \text{m}^{-3}$) roughly 30, which agrees well with the present investigation. In bottle experiments with sewage or nutrient enrichment of Öresund water, the C/chl *a* quotient based on chemical analysis of particulate matter was about 25 for both *Phaeodactylum tri-cornutum* and mixed (natural) phytoplankton populations (Granéli 1981).

Many authors have dealt with the C/chl *a* ratio of planktonic algae, both in cultures and under natural conditions (for references see de Jonge 1980). Reported values range from less than 10 to several hundred. Some of the variation is probably due to differences in analytical methods. POC is either determined chemically after filtration or through conversion of planktonic biovolume to organic carbon with appropriate factors (Banse op. cit., Strathmann 1967).

C/chl *a* quotients based on chemical determination of POC are compared in Tab 3. The examples include measurements on single species (batch) cultures, mixed enriched phytoplankton populations and natural plankton communities (using the regression method). The range of the values is about 20:1 to 110:1. None of these values refer to extremely nutrient-limited organisms. More extreme values have been reported from chemostat experiments (Kredel op. cit.), and it has been shown that the C/chl *a* ratio is inversely related to growth (dilution) rate (Laws and Wong 1978, Perry 1976). In slowly growing, nutrient-limited natural phytoplankton populations one would therefore expect a high

TAB 3. POC/CHL α QUOTIENTS FOR NATURAL PHYTOPLANKTON COMMUNITIES AND ALGAL CULTURES.

A REGRESSION METHOD - NATURAL UNENRICHED	POC/CHL α	REFERENCE
ABERDEEN BAY MAY - JULY	85	STEELE AND BAIRD, 1961
" " AUG - OCT	47	" " " "
LOCH NEVIS	74	STEELE AND BAIRD, 1962 A
LOCH CRERAN MEAN OF 41 VALUES	26	TETT ET AL., 1975
PERUVIAN COAST UPWELLING RANGE 39-64	$\bar{x}=41$	LORENZEN, 1968
B NATURAL PHYTOPLANKTON COMMUNITIES		
PLASTIC SPHERE <i>in situ</i> , ENRICHED, AT CHL α MAX	22	McALLISTER ET AL., 1961
PLASTIC BAG <i>in situ</i> , ENRICHED, AT CHL α MAX	NO ₃ 61	GRANÉLI THIS INVEST ^{x)}
(PN1) PNO ₃	45	" " "
(PN2) PNO ₃	48	" " "
SHIPBOARD CULTURE, UNENRICHED	43	EPPLEY ET AL., 1971
" " , ENRICHED	NO ₃ 90	" " " "
STATIONARY PHASE	NH ₄ 112	" " " "
" " , "	UREA 96	" " " "
LOG PHASE, "	NO ₃ 46	" " " "
" " , "	NH ₄ 58	" " " "
" " , "	UREA 77	" " " "
C MONOCULTURES		
<i>Nannochloris</i> AT CHL α MAX	35	STEELE AND BAIRD, 1962 B
<i>Skeletonema</i> AT CHL α MAX	28	" " " "
<i>Navicula pelliculosa</i> , SYNCHRONOUS CULTURE, SI-STARVATION	24-30	COOMBS ET AL., 1967
<i>Ditylum brightwellii</i> , TANK (BATCH)	NO ₃ 20	STRICKLAND ET AL., 1969
" " " "	NH ₄ 22	" " " "
<i>Phaeodactylum tricornutum</i> , BATCH, CONTINUOUS LIGHT, ENRICHED	24	GRANÉLI, 1981

x) CALCULATED FOR NET INCREASE IN CHLOROPHYLL α AND POC FROM DAY 1 TO DAY WITH MAXIMAL CHLOROPHYLL α CONCENTRATION.

quotient. In the oligotrophic waters of the central North Pacific Ocean Sharp et al. (1980) found a slope for the POC - chlorophyll α regression line of 242. However, only 36 % of the POC was estimated to be phytoplankton carbon, giving a true quotient of 87:1. The high POC/chl α quo-

tients recorded initially in the bags may indicate both slow growth rate caused by nutrient limitation and detrital influence. Edler (1977) calculated from plasma volumes that the C/chl *a* quotient for phytoplankton in the Öresund during summer was about 60:1 (with large variations). Based on an equation by Eppley (1972), Gargas et al. (1978) concluded that the C/chl *a* quotient in phytoplankton during the summer period in the Öresund was higher than 60. These relatively high figures are probably indicative of nutrient depletion.

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BIOASSAY EXPERIMENTS IN THE FALSTERBO CHANNEL.
NUTRIENTS ADDED DAILY.

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ABSTRACT

In situ enrichment bioassays were performed during the summers of 1978 and 1979 in the Falsterbo Channel, south Baltic Sea. Phosphorus and/or nitrogen was added daily for up to two weeks to 200 l polyethylene bags with unfiltered surface water. Additions of nitrogen or nitrogen plus phosphorus invariably increased the biomass and ^{14}C fixation of phytoplankton. Phosphorus additions had no such effects. Phytoplankton species which reacted most strongly to the enrichment were *Aphanothece* sp., *Nodularia spumigena*, *Skeletonema costatum*, *Nitzschia closterium*, *Chaetoceros wighamii* and *Oocystis* sp. The mean C/Chl *a* quotient was around 70 for chlorophyll *a* values below $6 \text{ mg} \cdot \text{m}^{-3}$ but decreased to about 30 for chlorophyll *a* values above 10.

INTRODUCTION

Nitrogen has often been regarded as the most important limiting nutrient for phytoplankton production in marine waters (Goldman 1976). In the Baltic Sea phosphorus was felt to be the most limiting nutrient for primary production by Fonselius (1977), while Sen Gupta (1972) proposed nitrogen. In the Bothnian Bay phosphorus seemed to be in short supply in the central areas while nitrate was depleted by early summer in coastal waters (Alasaarela 1979). With respect to the Öresund, eutrophication has usually been discussed in terms of phosphorus (see ref in Edler 1977), partly because of the lack of reliable long-term measurements of nitrogen.

In earlier studies in the Öresund and adjacent waters (Granéli 1978, Granéli in prep), it was shown that nitrogen additions to natural phytoplankton communities usually stimulated phytoplankton growth while phosphorus rarely did so. In these enrichment bioassays nutrients were added only at the beginning of the experiments.

In the present *in situ* bioassays, nutrients were added daily to plastic bags to simulate a permanent increase in the fertility of the water. Because of rupture of the bags, the experiments could not be run for more than about two weeks. Even so, this approach better corresponds to a real situation of nutrient enrichment than only initial nutrient addition. Chlorophyll *a*, ^{14}C fixation, phosphorus and nitrogen uptake and changes in the phytoplankton populations were studied in the various bags.

MATERIALS AND METHODS

The experiments were carried out in the Falsterbo Channel on the southwest coast of Sweden. The southern end of the chan-

nel is widened and protected by breakwaters, which makes it an excellent location for *in situ* experiments.

Three experiments were performed during the following periods: August 23 to September 4, 1978 (12 days), June 23 to July 7, 1979 (15 days) and July 1 to July 7, 1979 (7 days). 200 l double polyethylene bags (0,1 mm thick) were filled with 150 l unfiltered surface water. The bags were fixed to a wooden frame with styrofoam floats which held the open ends about 30 cm above the surface. The frame was anchored to the bottom at 4 m depth. A roof of polyethylene covered the construction. The water in the bags was mixed before sampling with a 30 cm diam. Secchi disc.

Four different bags were used in the enrichment bioassays: 1) control (C) containing untreated surface water, 2) one bag with phosphorus (P) added as KH_2PO_4 , 3) one bag with nitrogen (N) added as NaNO_3 and 4) one bag with both phosphorus and nitrogen added (PN).

In the bioassays lasting for 12 and 15 days, 20 l water was removed from each of the bags daily and 20 l of unfiltered surface water from the experimental site was added. New spikes of P and N were added with the water. In the experiment lasting 7 days, 5 l of water were taken out daily, but only nutrients and no new water were added.

The phosphorus and nitrogen additions to the bags were:

	Phosphorus ($\mu\text{g} \cdot \text{l}^{-1}$)		Nitrogen ($\mu\text{g} \cdot \text{l}^{-1}$)	
	Initial	Daily	Initial	Daily
12 day exp. (1978)	24	2.2	400	16
15 day exp. (1979)	35	11	500	154
7 day exp. (1979)	35	11	350	154

Water was filtered through Whatman GF/F filters for chlorophyll *a* analysis. Chlorophyll *a* was determined with the spectrophotometric equations of Jeffrey and Humphrey (1975) (see also Edler 1979). For measurement of H^{14}CO_3 assimilation 30 ml glass bottles with water from the bags were inoculated with 4 μCi -bicarbonate and incubated close to the bags at 0.3 m depth for 2 hours. The contents of the bottles were filtered and ^{14}C activity on filters was determined with Geiger-Müller equipment. ^{14}C activity was transformed to $\text{mg C}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ using measurements of light, pH, temperature and salinity (Gargas 1975). Light was measured at Sturup, about 30 km NE of the Falsterbo Channel.

Nutrients ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$) were analysed on unfiltered samples according to the methods in Carlberg (1972).

Water samples (150 ml) were preserved with Lugol's solution for identification and enumeration of the most frequent phytoplankton species. Nomenclature followed Hendey (1974) and Parke and Dixon (1976). The algae were counted in an inverted microscope, and numbers were transformed to plasma volume (PV) using size measurements and formulae in Edler (1979). Plasma volume was transformed to cell carbon using the factor 0.11 (Strathmann 1967), and C/Chl*a* quotients were calculated (mg/mg).

RESULTS

Weather conditions during the experiments.

August 1978 was colder than normal and had lower than normal rainfall. Weather changed to overcast with much precipitation, however, during the last week in August when the bioassay experiment was performed. This continued through the first week in September.

June 1979 was sunny and warm until June 22, when the weather changed to cold and overcast. The weather worsened during the first week in July. Thus weather conditions during both years' experiments were similar, i.e. cold and overcast with rain.

Water temperature during the experiments in 1978 varied between 10 and 18°C and in 1979 between 15 and 19°C.

Chlorophyll *a* (Fig. 1 a-c).

Initial chlorophyll *a* concentrations were 1.65, 0.68 and 1.38 mg·m⁻³ in the water used for the experiments a, b and c, respectively. Control and phosphorus bags showed the lowest chlorophyll *a* values in each experiment. There was an increase in chlorophyll *a* even in these bags, however. Additions of nitrogen alone gave maximal chlorophyll *a* values of 2.4, 7.9 and 3.6 times the initial values for a, b and c. Phosphorus and nitrogen added together gave the highest maximal chlorophyll *a* values in all 3 bioassays: a=3.7, b=39 and 52 (2 peaks) and c=11 times higher than the initial concentrations.

Chlorophyll *a* concentrations increased even in the control bags. In experiment b the increase was from initial value 0.68 to 2.65 mg·m⁻³ on the last day of the experiment. In the other bioassays the increase was less marked. In bags with phosphorus additions chlorophyll *a* concentrations were similar to or on some occasions even lower than concentrations in control bags.

Maximal chlorophyll *a* concentrations in the bags with only nitrate added were similar for the three bioassays, 4-5 mg·m⁻³, in spite of differences in the amounts of added nitrogen. When phosphorus was also provided, chlorophyll *a* con-

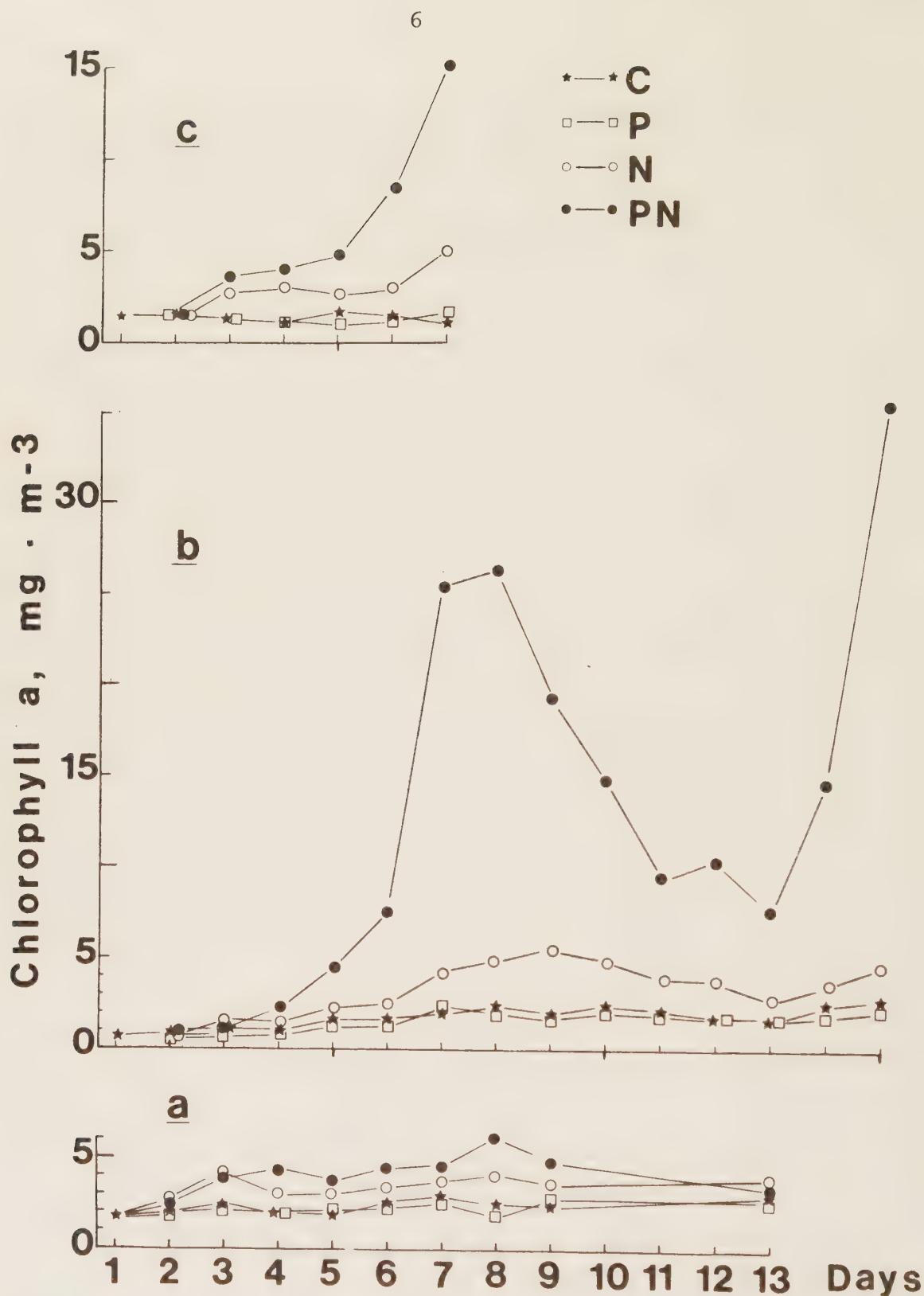


Fig 1- Chlorophyll *a* concentrations for the three experiments. a= August 23 to September 4, 1978, b= June 23 to July 7, 1979 and c= July 1 to July 7, 1979. C= control bag, P= phosphorus addition; N= nitrogen addition, PN= phosphorus plus nitrogen addition.

centrations reached about $30 \text{ mg} \cdot \text{m}^{-3}$ in experiment b and about $15 \text{ mg} \cdot \text{m}^{-3}$ in experiment c. In experiment a, on the other hand, maximal chlorophyll *a* concentration in the PN bag was only about $5 \text{ mg} \cdot \text{m}^{-3}$.

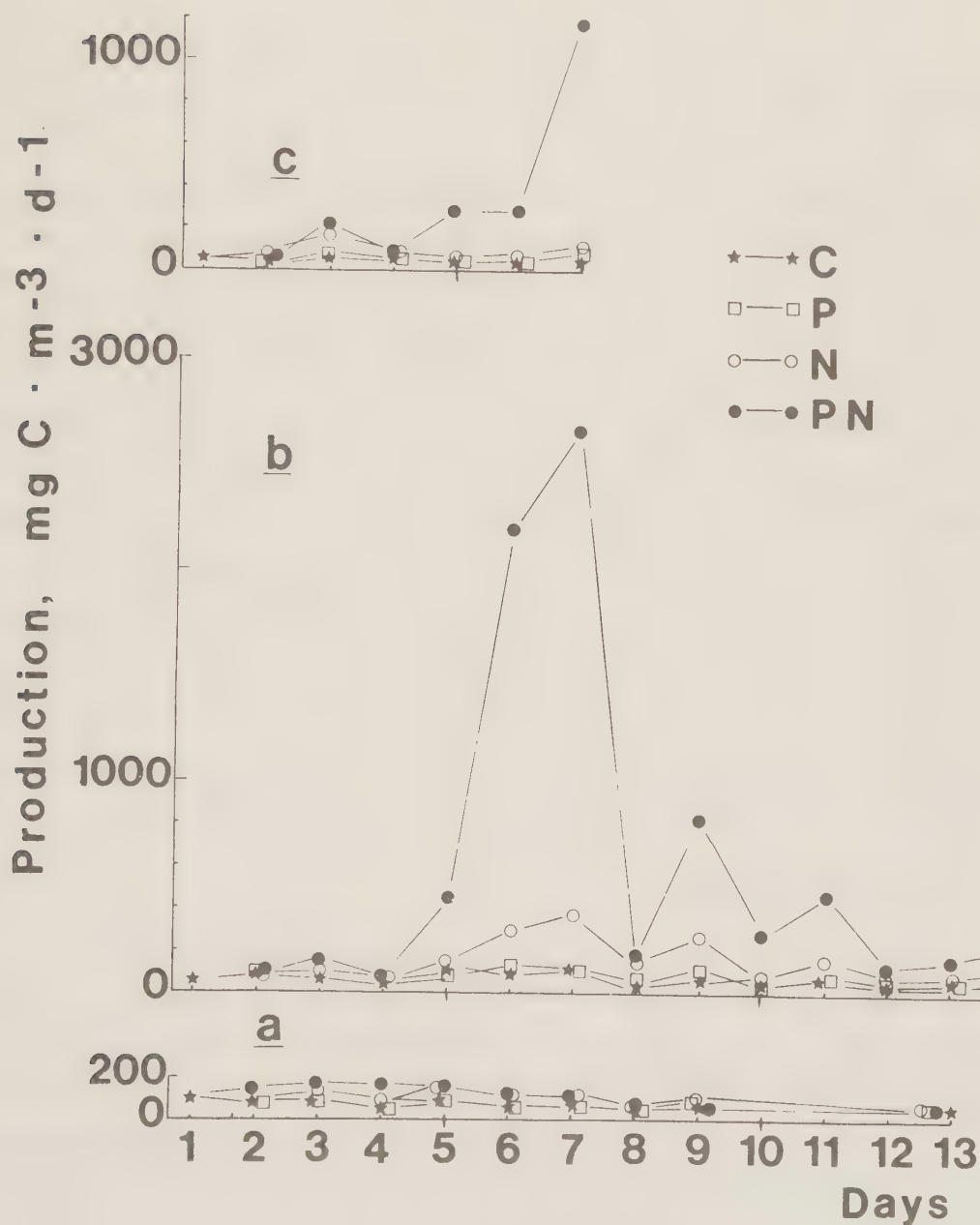


Fig 2- Primary production per day of bag water incubated outside the bags at 0.3 m depth. For identification of experiments a, b and c and symbols see Fig. 1.

Primary production (Fig. 2 a-c).

Initial ^{14}C -fixation in the water used for the experiments was 100, 54 and 58 $\text{mg C}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ for experiments a, b and c, respectively. Primary production followed a trend similar to that of chlorophyll *a* in all 3 experiments.

Phosphorus (Fig. 3 a-c).

Initial concentrations of inorganic phosphorus ($\text{PO}_4\text{-P}$) in the water used for bioassays were 4.1, 5.8 and 5.0 $\text{mg}\cdot\text{m}^{-3}$ (a, b and c, respectively). Control and nitrogen bags behaved similarly and showed only small changes in phosphate concentration. In all three experiments there was a slight decrease initially, and in experiment a phosphate concentrations in the C and N bags increased towards the end of the experiment. In the P bags of experiments a and b, phosphate decreased towards the end of the experiments while in experiment c there was a continuous increase. In the PN bags there was a rapid uptake of phosphate, although concentrations approached the values in the C and N bags only in experiment b.

Nitrate (Fig. 4 a-c).

Nitrate was initially present in low concentrations, between 5 and 7 $\text{mg}\cdot\text{m}^{-3}$ for the three experiments. In the C and P bags nitrate concentrations remained more or less constant in all 3 bioassays. In the N bags different patterns were observed: in experiment a, nitrate decreased to low levels after about one week; in experiment b nitrate was stable around 500-800 $\text{mg N}\cdot\text{m}^{-3}$, while in c there was a continuous increase. In the PN bags nitrate was depleted after 5 and 7 days, respectively, in experiments a and b. In experiment c, nitrate in the PN bag increased in the same way as in

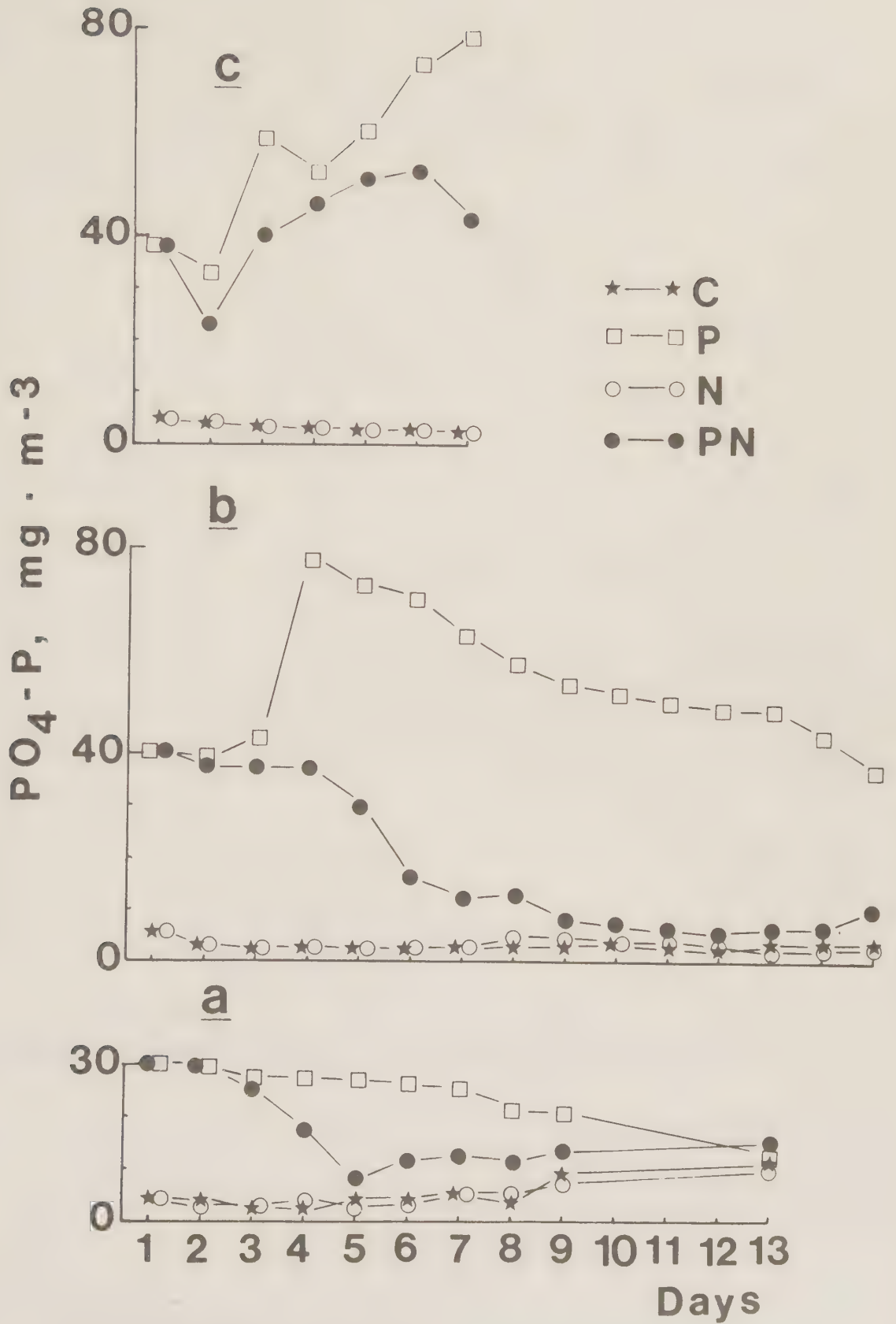


Fig 3- Inorganic phosphorus ($\text{PO}_4\text{-P}$) concentrations in the bags. For identification of experiments a, b and c and symbols see Fig 1.

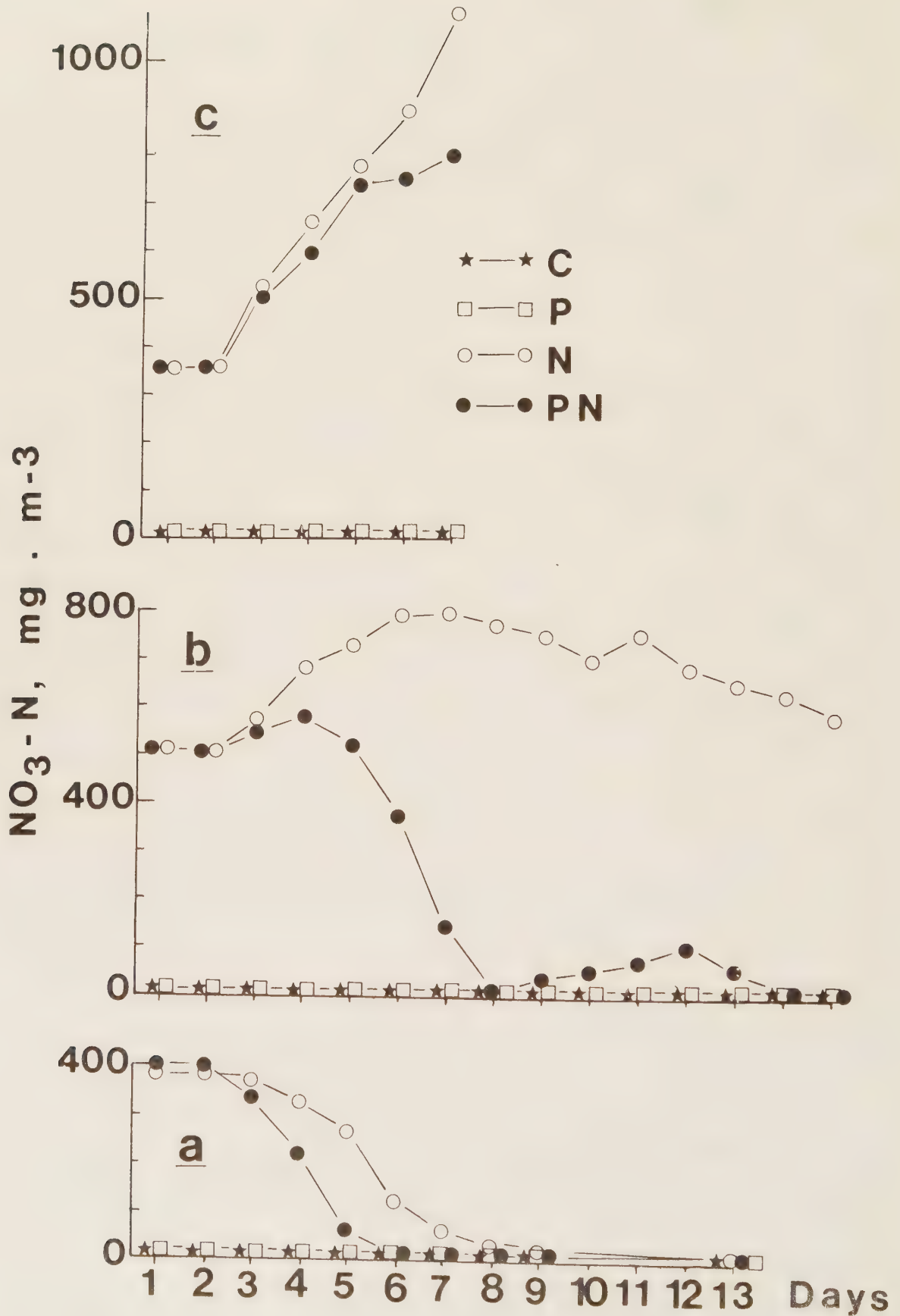


Fig 4- Nitrate nitrogen ($\text{NO}_3\text{-N}$) concentrations in the bags.
For identification of experiments a, b and c see Fig 1.

the N bag. However, experiment c was terminated on the 7th day when development of a chlorophyll *a* peak had begun. Nitrate consumption reached maxima of about 200 and 400 $\text{mg} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ in the PN bags of experiments a and b (Fig. 5 a-c). Nitrate consumption in the N bags peaked later than in the PN bags.

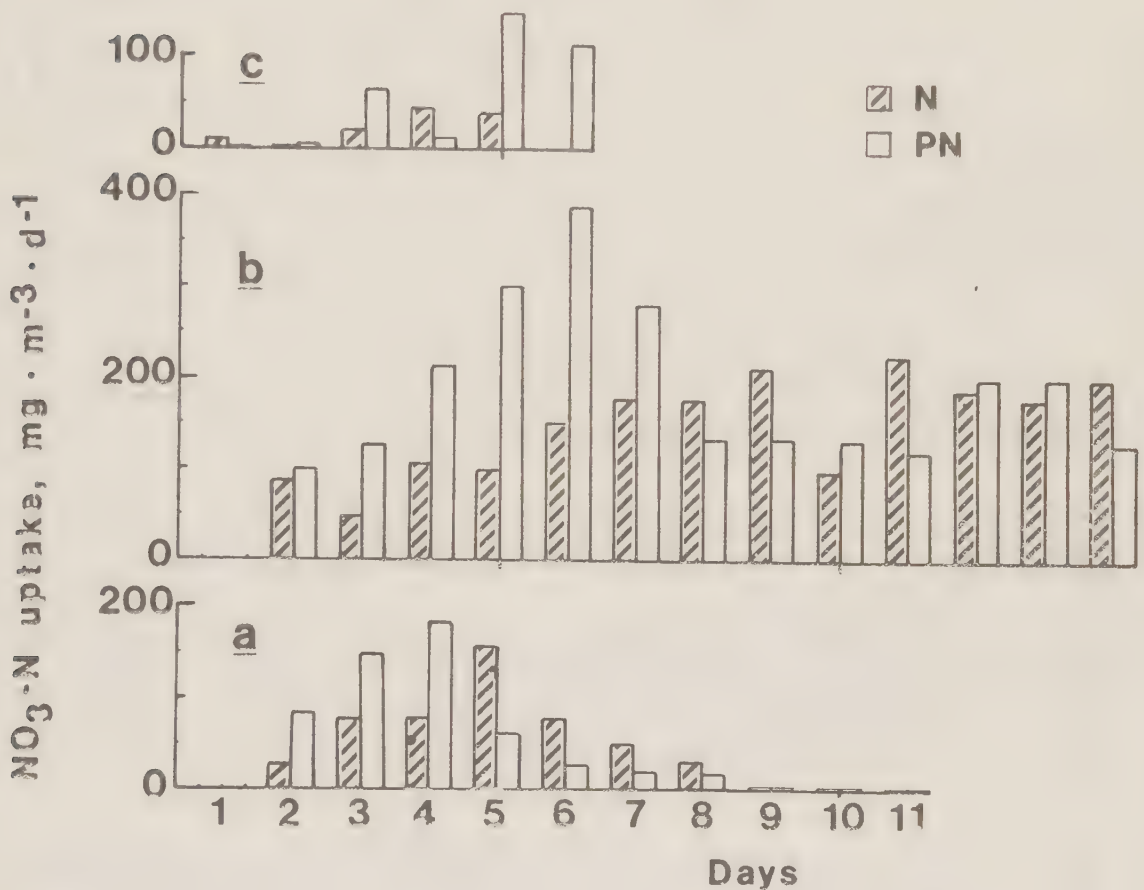
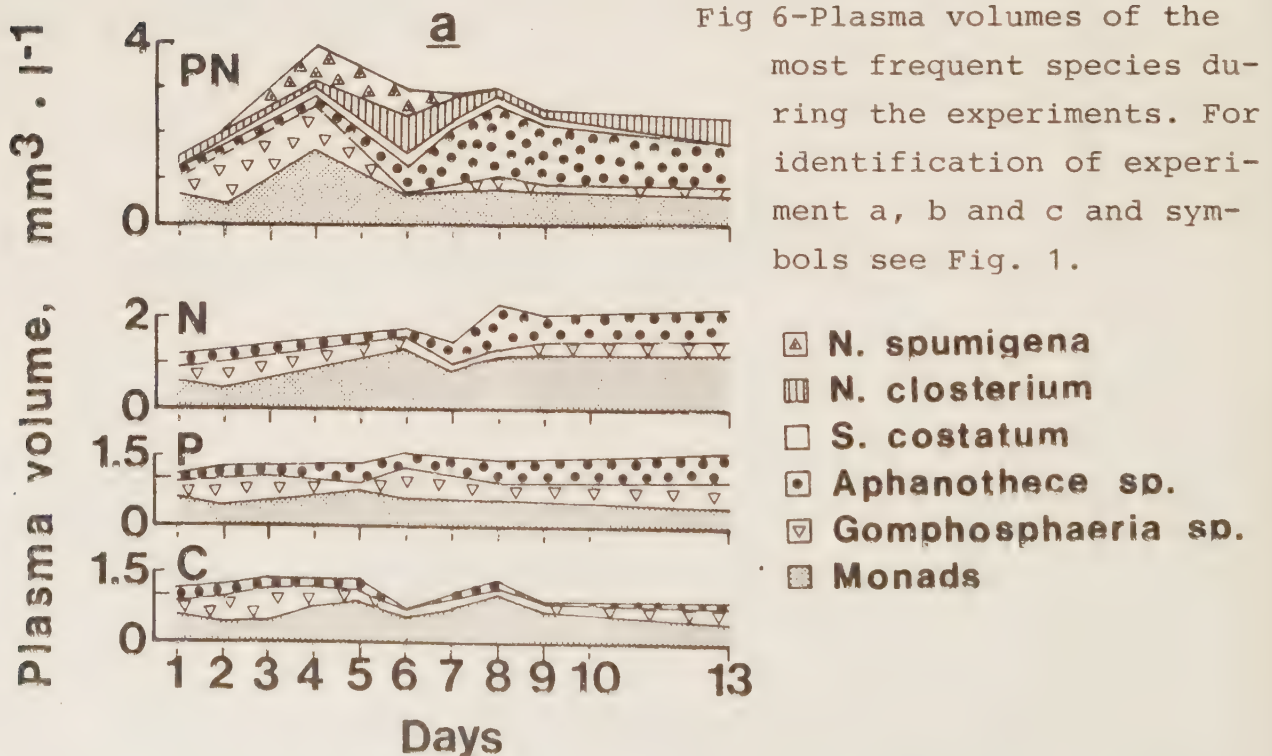
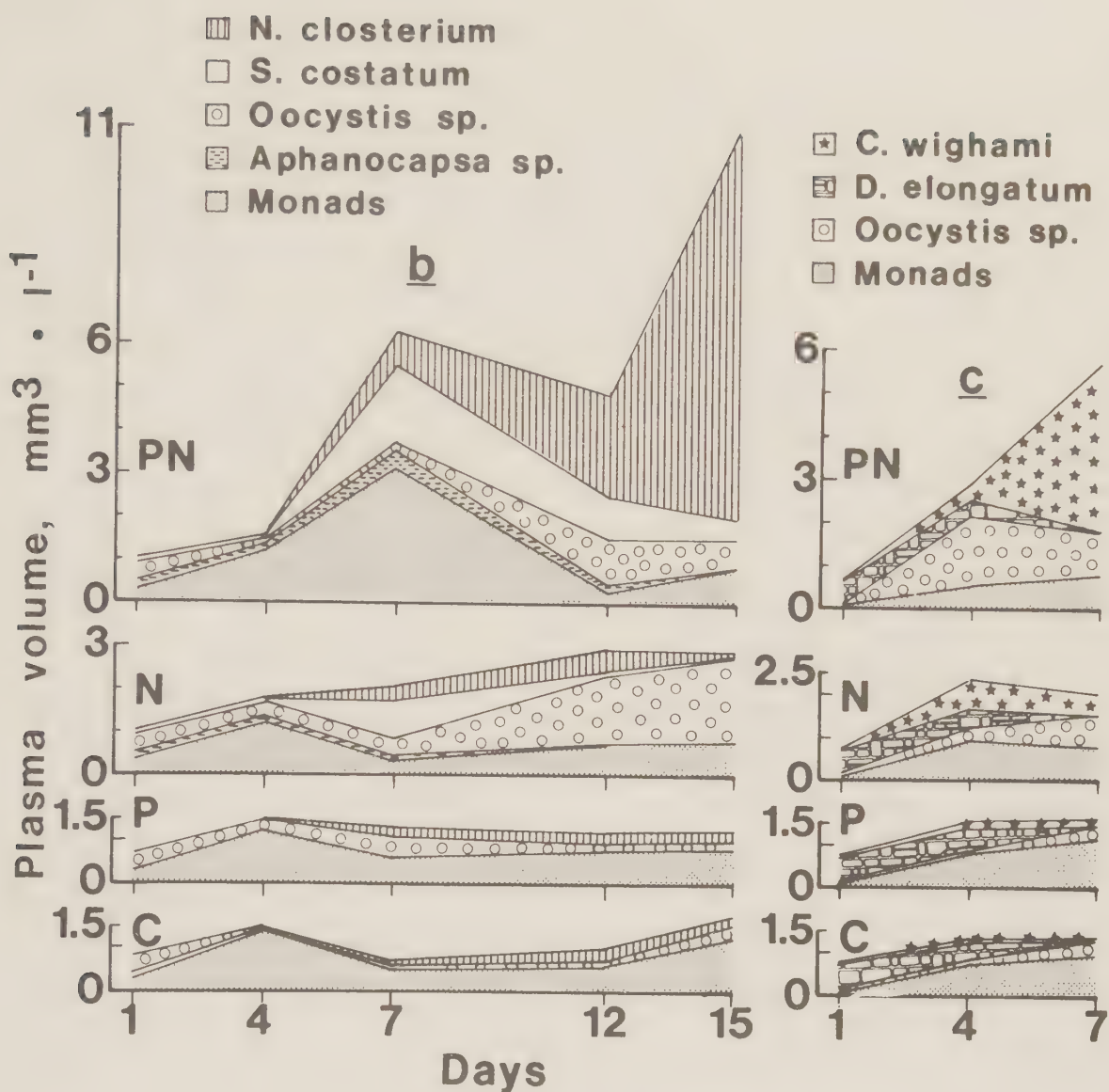


Fig 5- Uptake of $\text{NO}_3\text{-N}$ in the N and P bags calculated from concentration in the bags and daily additions. For identifications of experiments a, b and c and symbols see Fig. 1.

Phytoplankton composition and biomass (Fig. 6 a-c).

In experiment a the colonial blue-green alga *Aphanothece* sp. reacted most strongly to the addition of nutrients. The biomass of this species increased relative the control in all bags with nutrient addition. In the PN bag there was also an early peak of the nitrogen-fixing blue-green alga *Nodularia spumigena* and later of the diatom *Nitzschia closterium*. In experiment b, on the other hand, *N. closterium* and *Skeletonema costatum* reached the highest plasma volumes in the PN bag. *N. closterium* was present in small amounts in the other bags. The green alga *Oocystis* sp. developed strongly at the end of the experiment, especially in the N bag. As in experiment a, monads contributed significantly to the total plasma volume in all bags. In experiment c the diatom *Chaetoceros wighami* and a green alga of the genus *Oocystis* responded positively to nitrogen and nitrogen plus phosphorus enrichment. "Monads" and *Diatoma elongatum* dominated the phytoplankton biomass in the C and P bags.





There was a clear dependence of chlorophyll *a* concentration on phytoplankton plasma volume (Fig. 7). However, the C/Chl *a* quotient was not constant. The value was roughly 70:1 for chlorophyll *a* values below $6 \text{ mg} \cdot \text{m}^{-3}$, but decreased to about 30:1 for high chlorophyll *a* values ($>10 \text{ mg} \cdot \text{m}^{-3}$). These high chlorophyll *a* values were found only in bags enriched with phosphorus and nitrogen (experiments b and c).

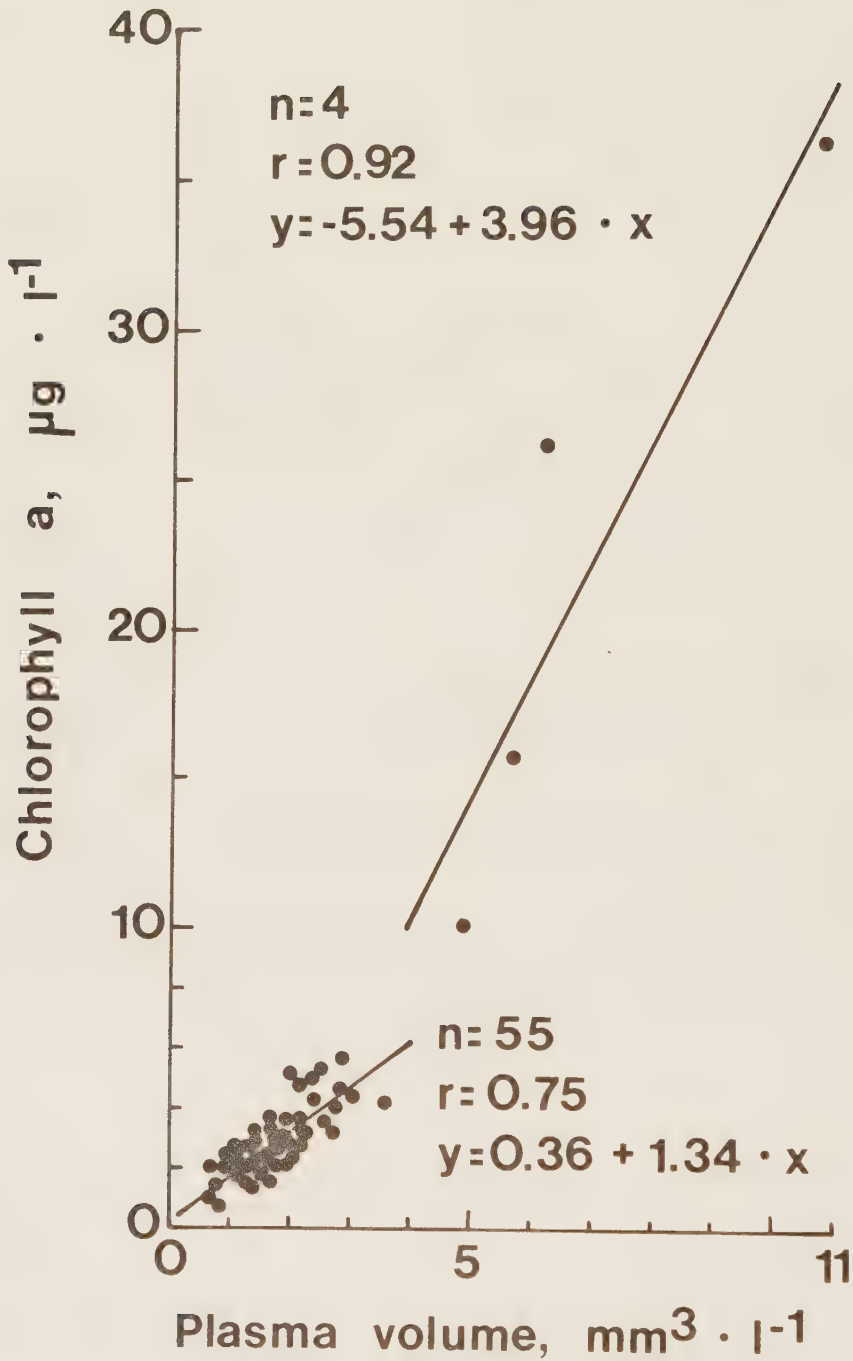


Fig 7- Relation between plasma volume and chlorophyll *a* concentration for all experiments and bags.

DISCUSSION

Enrichment experiments designed to test the "limiting nutrient concept" always deviate considerably from the natural situation, both with respect to time and space. To approximate the natural situation as well as possible, e.g. to simulate upwelling of nutrient-rich water or eutrophication of coastal waters through sewage discharge, there should be a continuous addition of nutrients to the test organisms and water renewal over a period of at least several weeks. All experiments could not be run this long because of destruction of the plastic bags, although runs for up to two weeks were achieved with bags intact.

As the water volume in the bags was about 150 l, and 20 l was exchanged per day in experiments a and b, the water renewal time was about one week. In the Öresund, where much of the water in the Falsterbo area is ultimately transported, surface water is renewed rapidly and mixed with Kattegatt water. A phytoplankton cell in the 0-10 m layer stays in the Öresund an average of 2-3 days (ÖVK 1979). With respect to water exchange the bioassay experiments (a and b) were conducted under conditions similar to those in the Öresund.

Addition of nutrients to the Öresund from municipalities and industries is substantial in spite of recent efforts to treat all sewage at least biologically (ÖVK op.cit.). The human population influencing the Öresund totals over 2 million, and there are fertilizer industries discharging large quantities of inorganic phosphorus. However, the actual increase in nutrient concentrations in the Öresund is limited by efficient mixing and rapid water renewal. Sustained "excess concentrations" of total N and P in the central part of the Öresund have been estimated to roughly 100 and 10 mg N and P.m⁻³, respectively (ÖVK op.cit.).

In experiments a and b, where water was exchanged daily, equilibrium concentrations of total N and P (assuming that nutrients were not lost through adsorption to the bags or denitrification) would be $16.5 \text{ mg P} \cdot \text{m}^{-3}$ and $120 \text{ mg N} \cdot \text{m}^{-3}$ (exp. a) and $82.5 \text{ mg P} \cdot \text{m}^{-3} / 1155 \text{ mg N} \cdot \text{m}^{-3}$ (exp. b). Thus the nutrient enrichment in experiment a was of the same order of magnitude as that observed in central Öresund. The nutrient additions in experiment b could only approximate those in shallow bays close to sewage outfalls (e.g. in the Lomma and Köge bays). Even the lower additions of N and P produced significant increases in phytoplankton biomass.

It is evident from the present experiments that nitrogen is more limiting to phytoplankton growth (in the sense of Liebig's law) than is phosphorus. Thus substantial increases in chlorophyll *a* and ^{14}C -fixation can be achieved through nitrogen enrichment alone. Unfortunately, eutrophication of the Öresund has often been discussed in terms of phosphorus enrichment. This is explained by a) a lack of reliable older data on inorganic and total nitrogen for the Öresund, and b) an uncritical application in brackish water ecology of ideas concerning nutrient limitation which were originally developed for fresh water. The practical/economic implications are obvious, e.g. with respect to sewage treatment.

If inorganic nitrogen is added in amounts over a few hundred $\text{mg} \cdot \text{m}^{-3}$, phosphorus becomes limiting. The generally steep increase in chlorophyll *a* when both nitrogen and phosphorus are added together indicates that other elements, e.g. trace metals, are not in short supply in the South Baltic.

Mean phytoplankton cell carbon/chlorophyll *a* quotients for low chlorophyll *a* values ($< 6 \text{ mg} \cdot \text{m}^{-3}$) were around 70, which is in agreement with summer ratios calculated by Edler (1977) for the Öresund from plasma volumes. When chlorophyll *a* increased to high values ($> 10 \text{ mg} \cdot \text{m}^{-3}$), which only happened in the PN bags of experiments b and c, plasma volume did not

increase proportionately. Thus C/Chl *a* ratios decreased to roughly 30, which is close to spring and autumn values for the Öresund (Edler op.cit.). In the spring nutrient availability in the Öresund is high, resembling conditions in the enriched bags. Variable C/Chl *a* quotients imply that chlorophyll *a* does not necessarily measure the increase in phytoplankton biomass during a nutrient enrichment (cf. Jonge 1980).

The experiments were made during a part of the year when monads and flagellates often compose a large part of the phytoplankton biomass in the south part of the Öresund. Monads were present in significant amounts in all the experiments but did not react strongly to nutrient enrichment. The greatest increase in biomass (plasma volume) was found for a few species of diatoms and blue-green algae. Among the former *S. costatum* is known to form blooms in the Öresund (Edler 1977). Although the experiments were of a rather short duration (up to 2 weeks), they show that stability with respect to phytoplankton species composition is not reached even with sustained nutrient enrichment.

In conclusion, the increase in primary production seen in the Öresund since 1930 (Gargas et al 1978, ÖVK 1980) has mainly been caused by increased availability of inorganic nitrogen. The hypothesis that inorganic phosphorus also limited phytoplankton production initially in this area can not be totally ruled out. However, phosphorus does not presently seem to limit phytoplankton growth, except perhaps during occasional late summer blooms of *Nodularia spumigena*.

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V

ALGAL ASSAY WITH SEWAGE AND ÖRESUND WATER - IMPLICATIONS
FOR SEWAGE TREATMENT OPTIMIZATION.

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ABSTRACT

Laboratory and *in situ* experiments were conducted to test the influence of sewage on algae growing in brackish water from the Öresund (SW Sweden) and from the Falsterbo Channel at the south entrance to the Öresund. Laboratory tests were made in 500 ml glass bottles using *Phaeodactylum tricornutum* or indigenous phytoplankton populations as test organisms. Öresund surface water was enriched with 1, 5 or 10 % raw or tertiary (phosphorus removal) sewage from a large treatment plant in Malmö, SW Sweden. Experiments were run approximately monthly from April 1976 to April 1977. Algal response to sewage enrichment was measured as chlorophyll *a* and POC (10th day) and ^{14}C uptake (4th day). *P. tricornutum* generally showed higher production and biomass values than the natural phytoplankton populations. POC and chlorophyll *a* were linearly correlated in both cases, with POC/chl *a* quotients of about 25. Production values and chlorophyll *a* concentrations were lower in bottles enriched with tertiary sewage than in bottles with raw sewage. Growth inhibition after sewage enrichment was seen only rarely. Inorganic nitrogen concentrations in raw and tertiary sewage were similar (about $20 \text{ mg N} \cdot \text{m}^{-3}$), while phosphate concentrations in raw sewage were about three times higher on an average (3.5 and $1.1 \text{ g P} \cdot \text{m}^{-3}$). However, chlorophyll *a* yield was reduced only about 30 % when raw and tertiary sewage additions were compared at the 1 % level. This is explained by the generally low inorganic N/P quotients in the Öresund (mean for the investigation period about 3).

In situ sewage enrichment experiments were performed in the Falsterbo Channel in June and August 1979 in 200 l transparent polyethylene bags. Primary, secondary and tertiary sewage from treatment plants in Lund and Genarp, SW Sweden, was added to unfiltered channel water to 1 or 5 % final concentrations. Chlorophyll *a*, ^{14}C fixation and dissolved inorganic N and P were measured daily in the bags for about 10 days. Background nutrient and chlorophyll *a* concentrations in the channel

were low. Sewage enrichment increased maximal chlorophyll *a* concentrations and assimilation numbers compared to control bags, with the highest stimulation from primary sewage. In bags with primary sewage phosphorus was in excess over nitrogen when chlorophyll *a* peaked. In bags with tertiary sewage inorganic phosphate was totally consumed while nitrate remained. Nitrate was not used by the algae until ammonia had reached low concentrations. Inorganic nitrogen and phosphate were utilized in highly variable proportions, low for primary sewage (<10) and high for tertiary sewage (>10). There were no constant proportions between nutrients consumed and chlorophyll *a* produced.

It is concluded that lack of inorganic nitrogen potentially limits phytoplankton development in the Öresund and in the adjacent south part of the Baltic. Nitrogen limitation is probably a natural phenomenon in the Öresund and not a result of discharge of sewage from the population of 2 million, although N and P concentrations have significantly increased in the central part of the strait. On the Swedish side of the Öresund sewage treatment plants have adopted phosphorus removal, while biological treatment is used on the Danish side. Large amounts of phosphates are discharged to the north part of the Öresund by fertilizer industries. Although about 90 % of the phosphorus discharge could be eliminated, even this efficient sewage treatment would still not make phosphorus limiting for phytoplankton production in the central Öresund. Nitrogen sources are more difficult to control because of a large diffuse addition from heavily fertilized soils. Nitrogen removal in municipal sewage would significantly reduce potential phytoplankton biomass in the Öresund, however.

INTRODUCTION

Problems connected with wastewater disposal have increased rapidly during the present century all over the world as a consequence of urbanization, increased standard of living and industrialization. Originally, hygienic and aesthetic aspects of wastewater treatment were considered most important. Mechanical sewage treatment plants were built, often with chlorination. Later, adverse effects of the organic matter in effluents on the recipients were stressed. Biological treatment plants (usually with trickling filters or activated sludge basins) were constructed to reduce the load of oxidizable organic matter (BOD). Although these treatment plants are often highly effective (> 90 % BOD removal), problems in recipients continued. During the nineteen-fifties and -sixties aquatic biologists and sanitary engineers realized that the eutrophication of recipients by plant nutrients in secondary effluents had to be controlled. As a consequence most sewage treatment plants in Sweden were supplemented with a tertiary stage involving the removal of phosphorus through chemical precipitation with lime, iron salts or aluminium sulphate. Today about 80 % of the urban population in Sweden (which makes up 83 % of the total population) is connected to sewage treatment plants with chemical precipitation (Statens Naturvårdsverk 1979).

Cultural eutrophication is most easily detected in small recipients, e. g. lakes, where it usually has been associated with increased loading with phosphorus. It is nowadays agreed upon that inorganic phosphorus often limits primary production in oligotrophic and mesotrophic lakes (Schindler 1977, 1978), and that phosphorus can be made limiting in many lake recipients through removal in treatment plants. However, this was not generally accepted when the Swedish program for tertiary sewage treatment fa-

cilities was initiated in the nineteen-sixties. One can recall the pseudoscientific "limiting-nutrient controversy" in North America (Likens 1972) and the debate concerning the usefulness of banning detergents containing phosphates (Lund 1974).

In connexion with the rapid increase in tertiary treatment of municipal sewage in Sweden, a research program was started in order to investigate the effects of reduced phosphorus loads on recipients, in this case exclusively lakes (Forsberg 1972). Beneficial effects could be detected in some cases (Forsberg 1978, Forsberg et al. 1978a), although the response of the lake ecosystem appeared in many instances to be delayed because of the buffering capacity of nutrient-loaded sediments and the addition of nutrients from diffuse sources (Ryding and Forsberg 1976, 1977, Forsberg 1979).

Because of the larger volumes of water involved and the often more-rapid water renewal compared to lakes, eutrophication effects of domestic and industrial sewage are not as easily detected in coastal areas as in lakes (cf. however Lee et al. 1978). In archipelagoes and coastal bays situated close to large cities, severe eutrophication problems have appeared, however (Ryther and Dunstan 1971, Melvasalo and Viljamaa 1977). The Swedish coast contains extensive archipelagoes, both on the west and east coasts, and eutrophication is obvious in the vicinity of e. g. Stockholm (Waern 1973, Karlgren and Ljungström 1975) and Gothenburg (Björn-Rasmussen 1975, Söderström 1979). Whether eutrophication effects exist in the outer archipelagoes and the open Swedish waters (the Baltic, the Kattegat and the Skagerrak) is more uncertain. Anoxic conditions in deep waters of the Baltic leading to release of nutrients from the sediments and increased primary and secondary production could be connected to cultural eutro-

phication (Karlgrén 1978, Cederwall and Elmgren 1980). However, anoxic conditions and blooms of blue-green algae are also considered to be natural phenomena for the Baltic (Horstmann 1975, Hallberg 1978, Fonselius 1978, Niemi 1979). Thus the observed increase in surface concentrations of phosphorus in the central Baltic might not be caused by man (Fonselius 1980).

In the SW part of Sweden the coasts are open without archipelagoes and eutrophication should be limited. However, in the Öresund, which receives nutrient-poor water from the Baltic, there has been a noticeable increase in annual primary production since the first measurements during the nineteen-thirties and fifties (Edler 1977, Gargas et al. 1980). The Öresund receives sewage - directly and indirectly - from an urban area of more than two million people and agricultural drainage from the best and most heavily fertilized soils in Sweden. In spite of this massive discharge of nutrients, phytoplankton biomass is still relatively low (Edler op. cit.), which may be ascribed to the rapid water renewal and possibly to adverse effects of rapid salinity fluctuations on phytoplankton (Edler op. cit.). However, in shallow bays in the Öresund and north of the area there are signs of a marked increase in e. g. macroalgal production, and dinoflagellate blooms have been recorded. These conditions interfere with recreation and fishing (Fleischer et al. 1978, Edler pers. comm.).

During recent years several sewage treatment plants in the Öresund region have been furnished with phosphorus removal facilities; the most noteworthy example being the plant at Sjölundå which serves the city of Malmö with over 200 000 inhabitants (Fig 1). On the Danish side of the Öresund treatment facilities have been built to the secondary level, and no tertiary treatment is planned in the immediate future (Damgaard 1980).



Fig 1. Map of the Öresund area. Sewage was collected from the treatment plants in Lund, Malmö and Genarp. *In situ* experiments were done in the Falsterbo Channel. Water for laboratory bioassays was sampled at station 1 (near Barsebäck). Water for nutrient analyses was sampled south of the island of Ven (☆).

Although conditions in the Öresund have been extensively surveyed for several years (von Wachenfeldt 1975, Edler 1977, Gargas et al 1978, Öresundskommissionen 1980), no investigations have been initiated to evaluate the specific effects of tertiary sewage treatment. It is questionable whether chemical-biological surveys in such a large and complex recipient as the Öresund could detect these effects, since sewage treatment philosophy is not identical on the Swedish and Danish sides and phosphates in vast quantities are discharged from fertilizer industries in the northern part of the Swedish Öresund region (Boliden AB, Helsingborg). Present discussion concerning effects of phosphorus removal must be rather theoretical (cf. Hanerz 1971, Steemann Nielsen 1976), and nutrient removal or addition experiments in delimited laboratory or *in situ* microcosms are probably of greater immediate value than expensive surveys.

In previous enrichment experiments with Öresund water, it was shown that nitrogen additions almost universally stimulate phytoplankton growth in batch-type experiments, while phosphate additions rarely do so (Granéli 1980, Sundström 1980). According to the principle of Liebig, nitrogen could be designated the primary limiting nutrient in the Öresund. Phosphorus could be regarded as the secondary limiting nutrient for phytoplankton production, as simultaneous additions of both phosphorus and nitrogen generally gave the highest phytoplankton yield. Judging from these experiments the rationality of removing phosphate from sewage discharged to the Öresund (and probably also to the whole of the South Baltic) could be questioned.

To confirm the results obtained with pure nutrient solutions, phytoplankton assays with sewage/recipient water mixtures were performed both in the laboratory and *in situ*

in plastic bags. Sewage from different treatment plants, both raw and after mechanical, biological or chemical treatment, was added to Öresund water in different proportions. Chlorophyll *a* concentrations and primary production (^{14}C method) were monitored, as well as changes in inorganic nutrients (N and P). Results from these and previous experiments (Granéli 1978, op. cit.) are discussed in light of current knowledge about nutrient limitation in the sea and in the Baltic, nutrient and phytoplankton conditions in the Öresund, and present and possible future sewage treatment facilities in the Öresund region.

MATERIALS AND METHODS

1. Laboratory experiments

Surface water samples were taken from a station near the nuclear power plant of Barsebäck (Fig 1) on 11 occasions from April 1976 to April 1977. Water was immediately brought to the laboratory in Lund in plastic containers. Samples were then filtered through Whatman GF/C glass fiber filters (for the *Phaeodactylum* tests) or a 180 μm mesh nylon net to remove large zooplankters (for tests with natural phytoplankton communities).

Raw and chemically/biologically-treated sewage (precipitation with iron chloride followed by activated sludge treatment) was collected at the Sjölanda treatment plant in Malmö (Fig 1). The sewage was a mixture of samples taken every hour during a 24-hour period. Samples for different months were collected on different days of the week.

Sewage samples were filtered through Whatman GF/C filters and then mixed with Öresund water to final sewage concent-

rations of 1, 5 and 10 % by volume. High sewage concentrations were chosen in order to get a measurable growth response by the test algae. Calculated for net outflow through the Öresund and total sewage discharge, the dilution factor would be about 500 (see Discussion). Sewage is much less diluted with recipient water, however, in shallow bays of the Öresund, e. g. Lomma bay (Fig 1).

Spherical glass flasks of 500 ml capacity were filled with 400 ml sewage/Öresund water mixture. On some occasions nutrient enrichment experiments were run parallel to the sewage experiments. Öresund water was then enriched with different nutrient solutions in concentrations equivalent to 10 % Z8 medium (Källqvist 1975, see also Granéli 1978).

The *Phaeodactylum tricornutum* Bohlin innoculum was taken from an exponential phase stock culture growing in 10 % Z8 medium. The innoculum corresponded to an initial cell concentration of $0.5-1.5 \times 10^6$ cells $\cdot$ l⁻¹. Flasks were incubated at 20 ± 1 °C and $4 \cdot 10^{-2}$ mW $\cdot$ cm⁻² (approximately 4000 lux, 40 W Asea-Scandia cool-white fluorescent lamps, continuous light). The flasks were shaken once a day.

Algal growth response was measured as chlorophyll *a* on the 10th day of the experiment with the method of Strickland and Parsons (1968, see also below under *In situ* experiments), i. e. values are uncorrected for phaeopigments. Preliminary tests showed that the algae reached stationary growth phase by the 10th day, so the chlorophyll *a* values represent final biomass yield. As an additional measure of growth response, primary production was measured on the 4th day (¹⁴C method of Steemann Nielsen 1952). Earlier experiments showed that nutrient additions generally did not stimulate ¹⁴C uptake on the first two days after enrichment (Granéli 1978). Subsamples for primary production measurement were incubated in 30 ml Pyrex bott-

les with 4 $\mu\text{Ci NaH}^{14}\text{CO}_3$ for two hours at the same light intensity and temperature as the bioassay flasks. Radioactivity of filters was measured with a GM counter after fuming with formalin and HCl, and cpm was corrected for dark uptake and background. Cpm was transformed to $\mu\text{g C}_{\text{fixed}} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ using measurements of pH, temperature and salinity, and the formulae in Gargas (1975).

Phaeodactylum cell numbers were counted with a model 302 Celloscope particle counter (10th day). For some of the bioassays, particulate organic carbon (POC) was analyzed on an International Oceanography MSE Carbon Analyzer after collection on 25 mm $\varnothing$ Gelman AE glass fiber filters preignited for 2 hours at 400 $^{\circ}\text{C}$. Five parallel analyses were made for each sample (10th day).

Analyses of inorganic nitrogen and phosphorus were made on unfixed water collected in the central part of the Öresund (Fig 1). These samples were obtained on different dates than the start of bioassay experiments. Phosphate was analyzed according to Murphy and Riley (1962), ammonia: Solorzano (1969), nitrate: Wood et al. (1967) and nitrite: Bendschneider and Robinson (1952), with modifications in Carlberg (1972). The same methods were employed for analysis of sewage after filtration through a GF/C filter.

2. *In situ* experiments

During summer 1979 two sewage-enrichment experiments using natural plankton communities were performed in the Falssterbo Channel (Fig 1). The first experiment ran from June 16 to 23 (exp a) and the second from August 2 to 16 (exp b). Plastic bags of 200 l volume were filled with 150 l unfiltered surface water from the channel. Various types and concentrations of unfiltered sewage were added to the

bags. Bags were exposed in the channel hung from a wooden staircase-like frame (Granéli 1981).

In exp a mechanically and chemically (phosphorus precipitation) treated sewage from the Källby (Lund) and Genarp treatment plants was mixed with channel water in proportions 1 and 5 % by volume. In this experiment treated sewage from Källby was obtained both directly after the post-sedimentation stage and after the water had passed a series of shallow ponds. In exp b mechanically, biologically and chemically-treated sewage from the Källby plant was added to the bags to 1 % final concentration.

Källby receives sewage from the city of Lund (about 70 000 inhabitants, Fig 1), and phosphorus is removed through addition of FeCl_3 . Genarp (Fig 1) receives sewage from a population of about 2 500, and phosphorus is removed by precipitation with $\text{Al}_2(\text{SO}_4)_3$. Both treatment plants are dimensioned for 90 % P removal, i. e. effluent concentrations of less than $0.5 \text{ g P} \cdot \text{m}^{-3}$. During summer dosage of precipitation chemical is reduced in Källby and efficient P-reduction is only achieved after sewage has passed the ponds.

5 l water was removed daily from each bag after vigorous mixing with a Secchi disc. Analyses of nitrate + nitrite, ammonia and inorganic dissolved phosphorus were made immediately in duplicate as described above. Water for chlorophyll α analysis was filtered through Whatman GF/C glass fiber filters. The filters were air-dried (in the dark) and deep-frozen with silica-gel. After thawing, filters were homogenized in 90 % acetone with a Teflon-coated rotating homogenizer, and chlorophyll was extracted about 20 hours in the dark at about 5°C . Chlorophyll α was estimated spectrophotometrically according to the tri-chromatic equations of Strickland and Parsons (1968).

Primary production measurements were made as for bottle tests (see above), except that 30 ml samples were incubated *in situ* at about 30 cm depth near (outside) the bags for two hours (ca. 9 - 11 a.m.).

RESULTS

1. Laboratory experiments

Mean $\text{PO}_4\text{-P}$ concentration in raw sewage from the Sjölanda treatment plant was $3.5 \text{ g} \cdot \text{m}^{-3}$ for the 11 samples taken during April 1976 to April 1977. The corresponding value for chemically/biologically treated sewage was $1.1 \text{ g P} \cdot \text{m}^{-3}$. During May through October the tertiary sewage showed high phosphate concentrations (Fig 2). Otherwise, $\text{PO}_4\text{-P}$ in the final effluent was under $1 \text{ g} \cdot \text{m}^{-3}$, indicating acceptable treatment results. Concentrations of inorganic nitrogen ($\text{NH}_4 + \text{NO}_2 + \text{NO}_3$) did not differ greatly between raw and chemically/biologically treated sewage (mean concentrations of inorganic N 20.7 and $20.1 \text{ g N} \cdot \text{m}^{-3}$, respectively). Inorganic nitrogen in raw sewage was almost exclusively ammonia, while nitrate (including nitrite) was a significant part of the inorganic nitrogen in tertiary sewage, especially during summer (Fig 2). Both phosphate and inorganic nitrogen concentrations decreased during winter (November - March). The mean N/P ratio (w/w) was 6.0 for raw sewage and 18.9 for chemical/biological sewage. This quotient was around 100 for the tertiary effluent on several occasions, indicating efficient phosphorus removal.

Inorganic nitrogen (mainly nitrate) in the Öresund, started to increase in October (Fig 3, cf. Granéli 1978). A peak was reached at the end of October, followed by a sudden decrease to a low value at the end of November. Thereafter inorganic nitrogen increased to the highest value

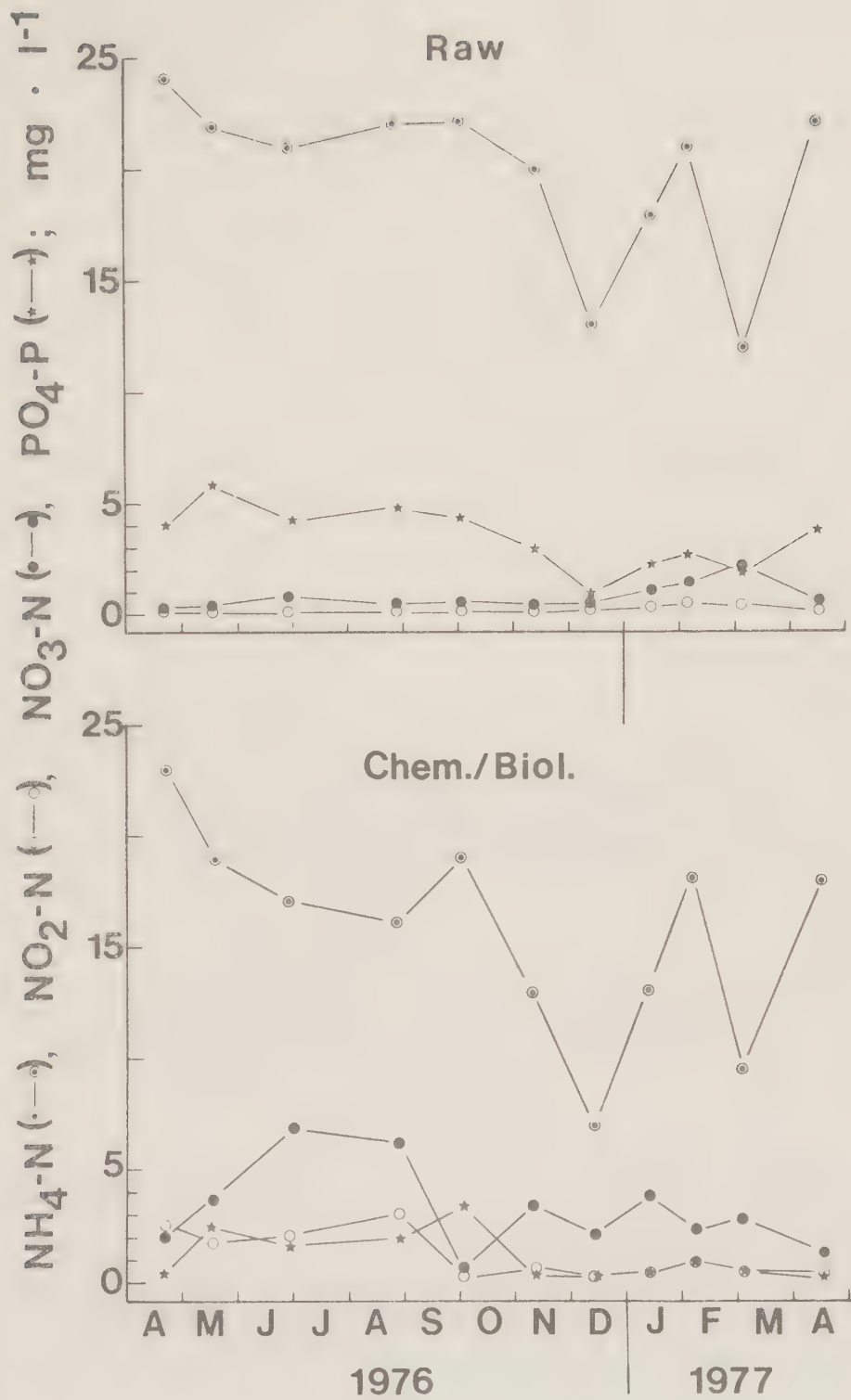


Fig 2. Concentrations of inorganic nitrogen and phosphate in sewage from Sjölanda (Malmö).

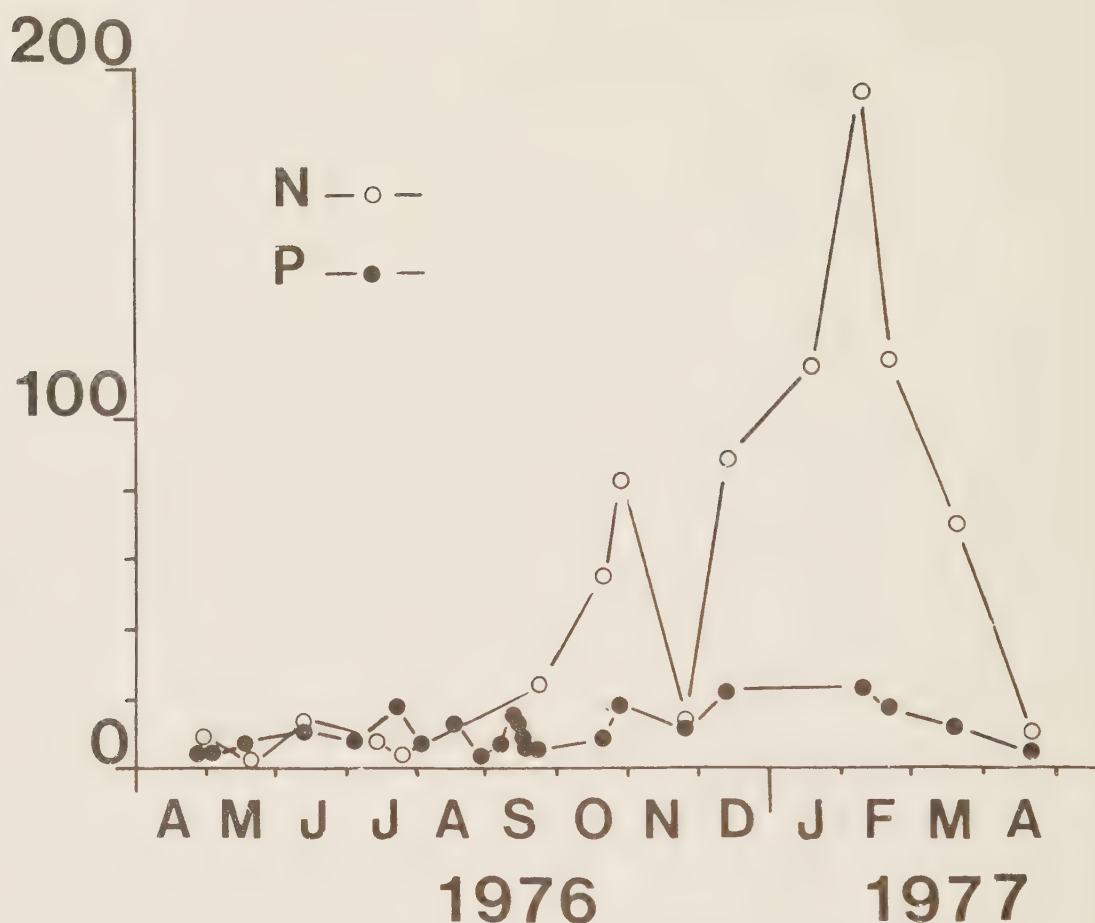
$\text{mg} \cdot \text{m}^{-3}$


Fig 3. Concentrations of dissolved inorganic nitrogen and phosphorus in central Öresund.

during the investigation period, about $200 \text{ mg} \cdot \text{m}^{-3}$, in February. By April less than $20 \text{ mg} \cdot \text{m}^{-3}$ inorganic N remained. The sudden decreases in inorganic nitrogen are connected to autumn and spring peaks in diatoms (Edler 1977). $\text{PO}_4\text{-P}$ concentrations changed irregularly during summer and autumn. High values were recorded during winter, with a decrease in spring simultaneous with the drop in inorganic nitrogen. The mean inorganic N/P quotient in central Öresund was 3.5 with extreme values of 0.1 and 8.2.

In the water used for bottle tests (sampled near the Barsebäck nuclear power plant) chlorophyll *a* peaks were recorded in November and April (Fig 4). The increases in chlorophyll *a* concentrations were likely connected to simultaneous decreases in inorganic N and $\text{PO}_4\text{-P}$ (cf. Fig 3).

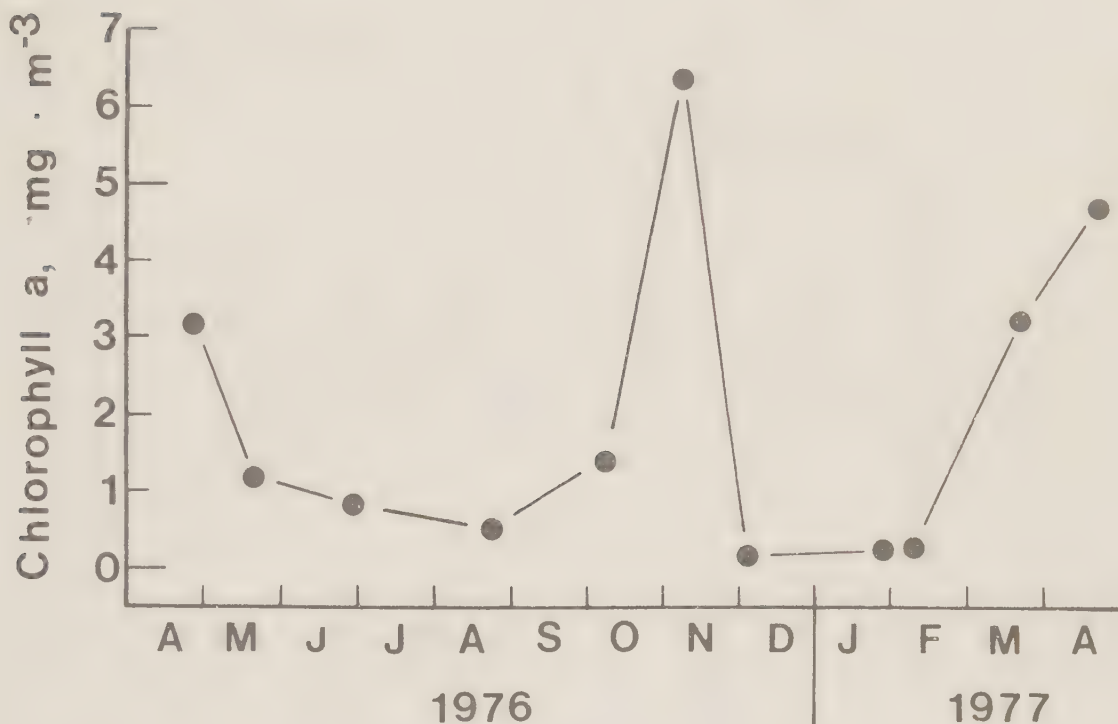


Fig 4. Initial concentrations of chlorophyll *a* in the laboratory bioassays.

High values for primary production in the natural population tests were recorded in October and March (Fig 5), i.e. when chlorophyll *a* concentrations were increasing most rapidly in the Öresund. Low ^{14}C fixation values were found in late spring and during winter. For the *Phaeodactylum* tests a more or less opposite pattern was found with high production during November through January (Fig 6).

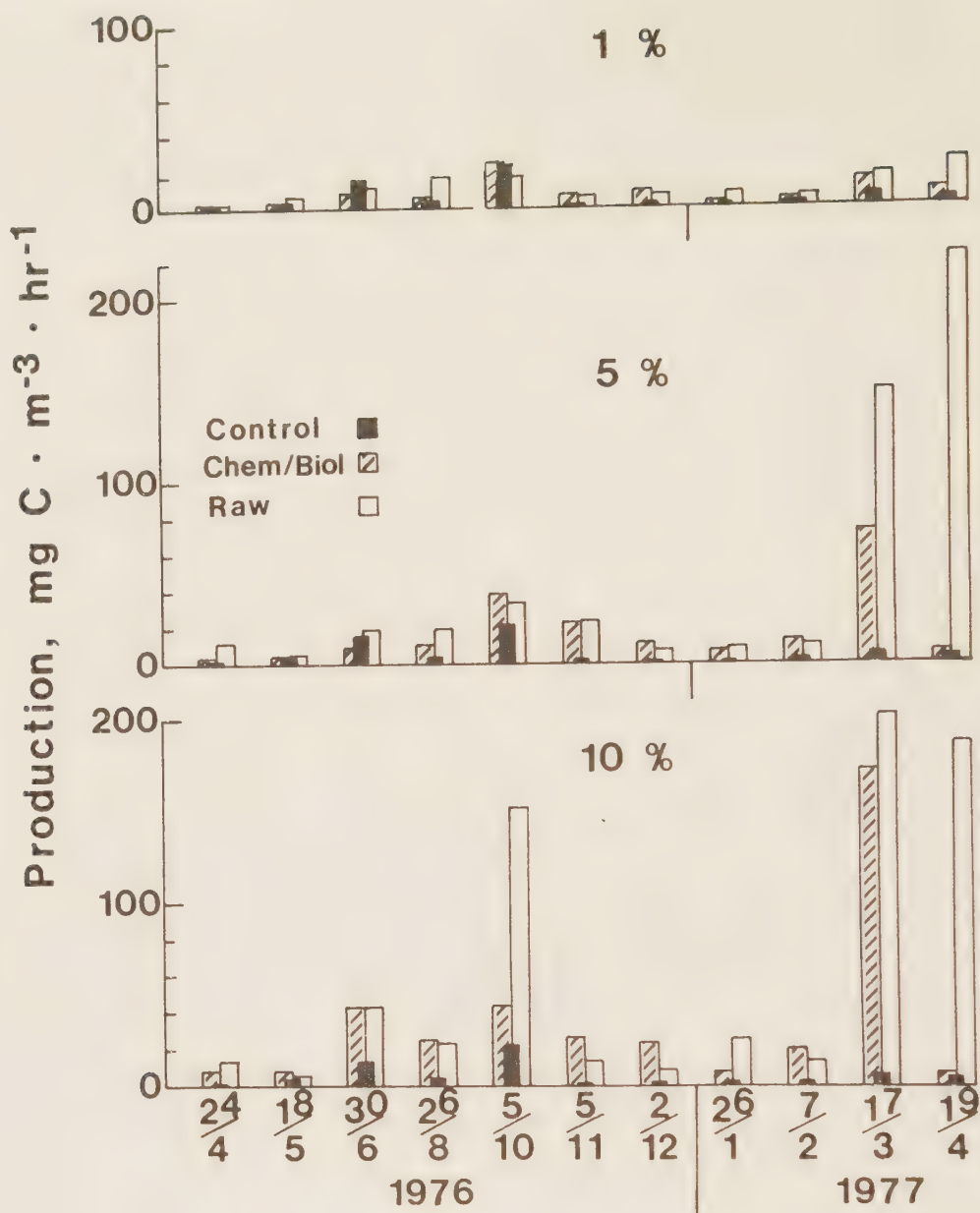


Fig 5. Primary production of natural phytoplankton communities on the 4th day of the bottle experiment. 1, 5 and 10 % raw and chemically/biologically treated sewage.

Production was generally higher for the *Phaeodactylum* tests than for the natural populations (Fig 7).

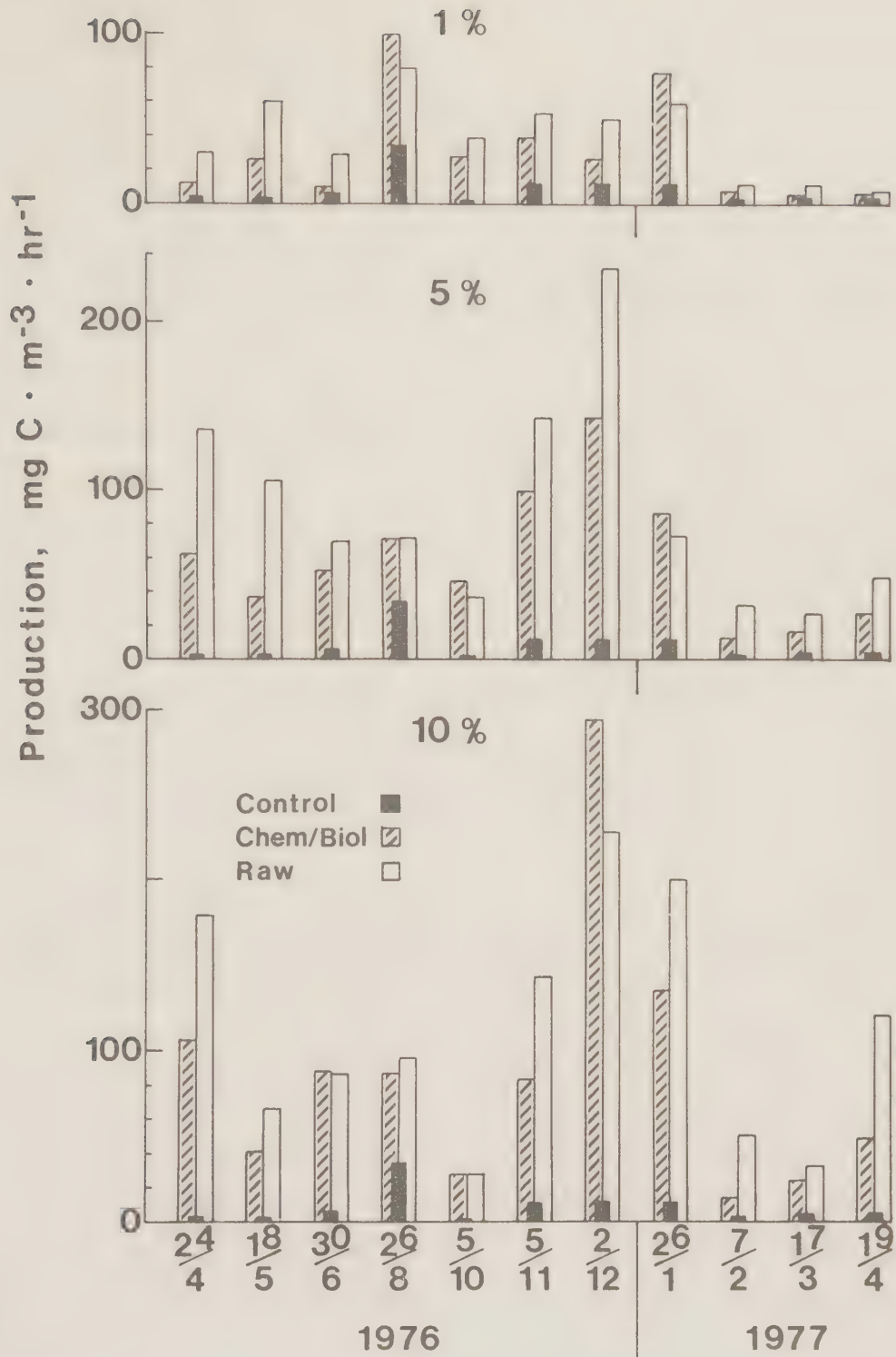


Fig 6. As Fig 5, but with *Phaeodactylum tricornutum* as the test alga.

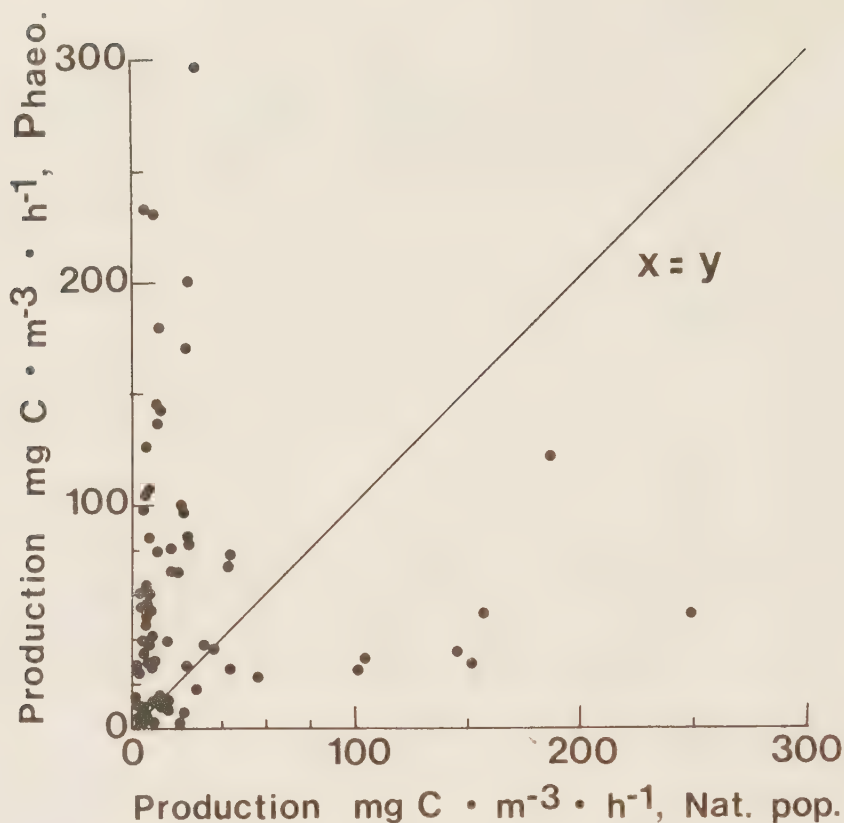


Fig 7. Production (^{14}C uptake) of *Phaeodactylum* compared to production of the natural communities.

Generally, the highest chlorophyll *a* concentrations were recorded for 10 % additions of raw or chemically/biologically treated sewage, both for the natural populations (Fig 8) and *Phaeodactylum* cultures (Fig 9). Maximal chlorophyll *a* values were on several occasions over $200 \text{ mg} \cdot \text{m}^{-3}$. With few exceptions, final chlorophyll *a* concentrations were higher for *Phaeodactylum* bioassays than for the tests with natural populations (Fig 10).

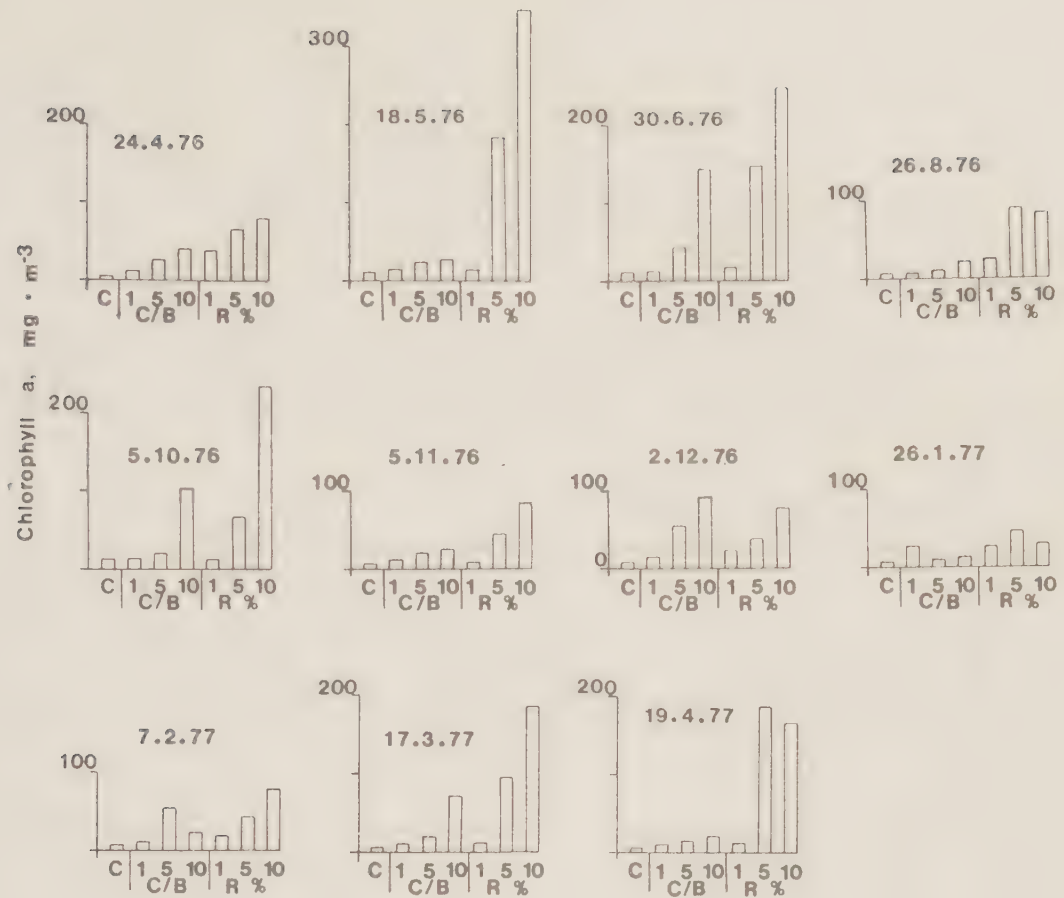


Fig 8. Chlorophyll produced by natural communities measured on the final (10th) day of the bottle experiment. C/B= chemically/biologically treated. R= raw. 1, 5 and 10 % sewage additions.

Signs of growth inhibition (lower chlorophyll *a* concentrations than in control bottles) were recorded for 1 % additions only and more frequently for natural populations than for *Phaeodactylum* (Tab 1 and 2). Chlorophyll *a* concentrations after 5 and 10 % additions of sewage were always higher than control values.

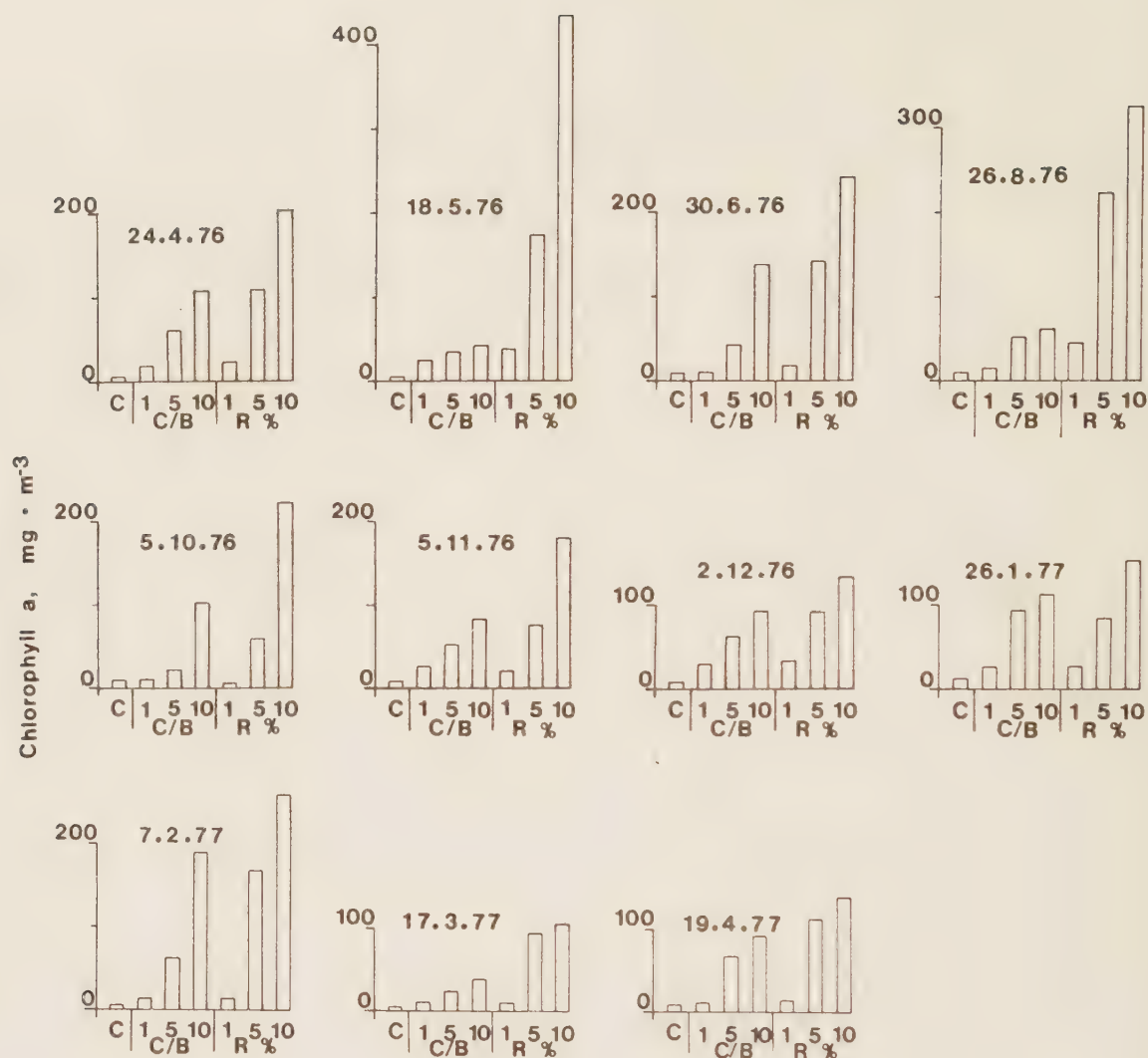


Fig 9. As Fig 8, but with *Phaeodactylum* as the test alga.

Both *Phaeodactylum tricornutum* and natural populations of Öresund phytoplankton grew better in mixtures with raw sewage than with tertiary sewage (Fig 11 and 12). The mean decrease in chlorophyll *a* yield between raw and tertiary 1 % sewage mixtures was roughly 30 % for both *Phaeodactylum* and natural populations. Algal growth was more or less proportional to the amount of raw sewage added (Tab 1 and 2). For chemically/biologically treated sewage,

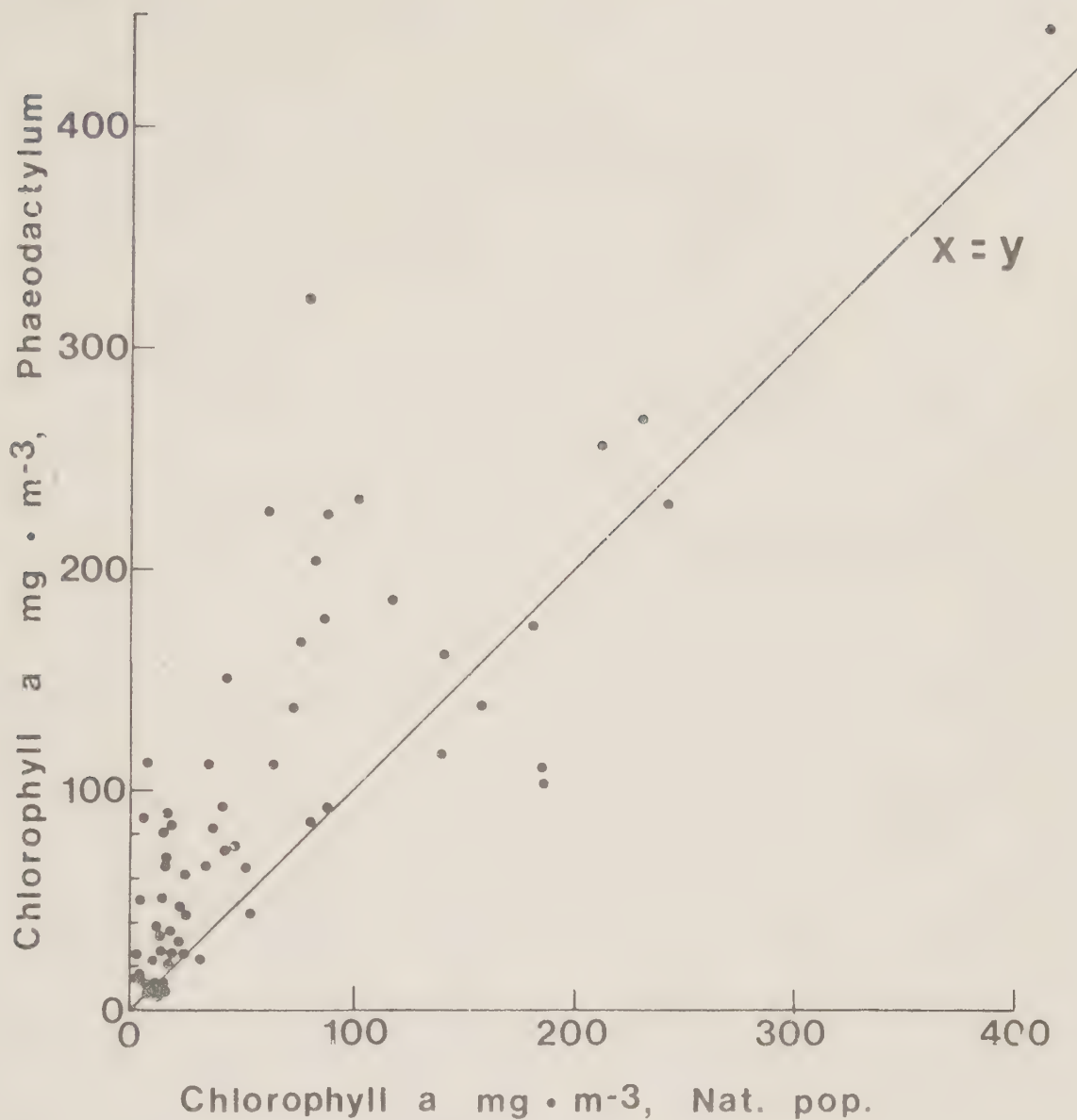


Fig 10. Chlorophyll *a* for the *Phaeodactylum* tests compared to chlorophyll *a* for the natural communities.

growth was retarded at higher additions, especially for natural populations.

TAB 1. NATURAL PHYTOPLANKTON COMMUNITIES. CHLOROPHYLL a CONCENTRATION AS PERCENTAGE OF CONTROL.

% SEWAGE ADDED	A	M	J	A	O	N	D	J	F	M	A	$\bar{X}$	EXPECTED ^{x)} :
1 CHEM/BIOL	198	122	87	98	97	172	172	317	177	184	145	158	
5 "	450	255	372	107	186	225	703	288	995	305	238	366	790
10 "	630	288	1303	375	997	232	1103	484	2107	1210	297	793	1580
1 RAW	579	138	146	466	50	45	261	244	260	218	170	235	
5 "	1096	2066	1341	1862	592	560	519	807	1323	1608	3230	1318	1175
10 "	1501	4235	2253	1677	2271	1169	988	1435	3770	3333	2766	2236	2350

x) EXPECTED VALUE IF CHLOROPHYLL a CONCENTRATIONS WERE STRICTLY PROPORTIONAL
TO AMOUNT OF SEWAGE ADDED.

TAB 2. PHAEODACTYLUM TRICORNUTUM, CHLOROPHYLL a CONCENTRATION AS PERCENTAGE OF CONTROL.

% SEWAGE ADDED	A	M	J	A	O	N	D	J	F	M	A	X	EXPECTED ^{x)} :
1 CHEM/BIOL	580	265	88	139	235	355	439	356	363	263	104	290	
5 "	3131	328	916	525	902	685	863	1151	3013	632	986	1194	1450
10 "	5358	415	1583	664	2503	1091	1150	1488	5403	1194	1280	2012	2900
1 RAW	1478	388	126	466	176	228	477	342	257	179	157	389	
5 "	6850	1773	1163	2330	2451	972	1081	972	4861	2681	1628	2433	1945
10 "	9021	4525	1278	3310	2883	2351	1835	2004	7543	2997	2048	3613	3890

x) EXPECTED VALUE IF CHLOROPHYLL a CONCENTRATIONS WERE STRICTLY PROPORTIONAL
TO AMOUNT OF SEWAGE ADDED.

Compared to 10 % Z8 addition (8.4 g N and $0.56 \text{ g P} \cdot \text{m}^{-3}$), 10 % addition of raw sewage (mean for five series 2.0 g N and $0.4 \text{ g P} \cdot \text{m}^{-3}$) gave higher (natural populations, Fig 13) or similar (*Phaeodactylum* tests, Fig 14) chlorophyll a concentrations when only P plus N were included. With additions of iron and EDTA together with P and N. *Phaeodactylum tricornutum* reached higher chlorophyll a concentrations.

than those obtained after 10 % addition of raw sewage. A 10 % addition of tertiary sewage (2.1 g N and 0.17 g P·m⁻³ added on an average) usually had less growth-stimulating effect than a 10 % Z8 addition. Nitrogen was the most stimulating nutrient, except for the natural populations in August (Fig 13).

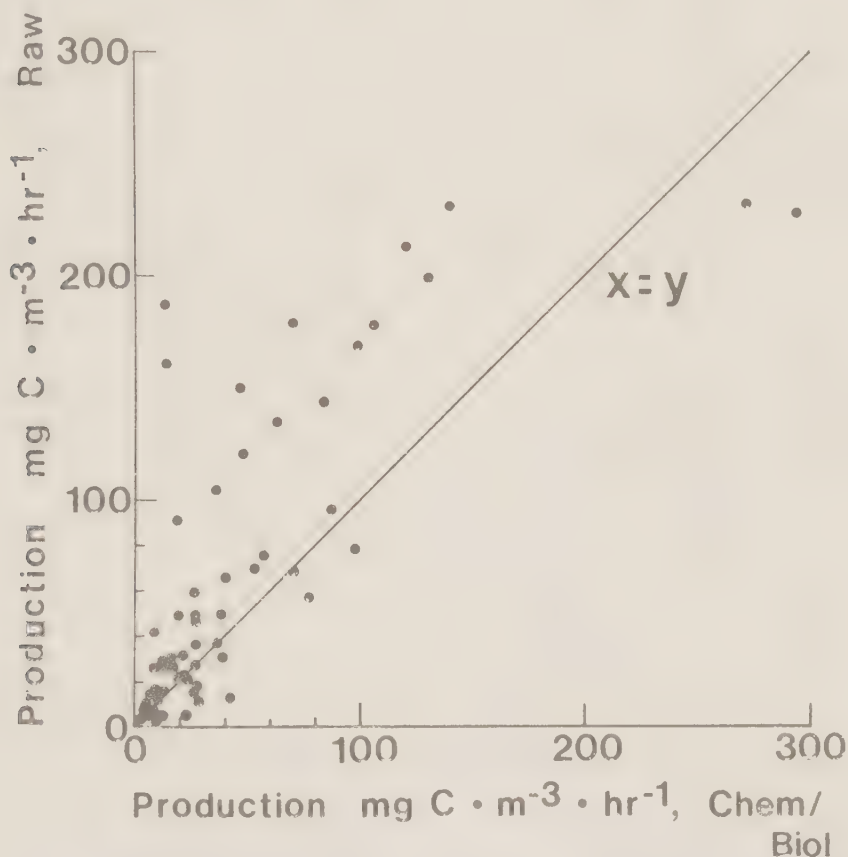


Fig 11. ¹⁴C uptake in bottles with raw sewage compared to ¹⁴C uptake after additions of chemically/biologically treated sewage (both natural communities and *P. tricornutum*).

There was a good linear correlation between particulate organic carbon (POC) and chlorophyll *a* for both natural populations and *Phaeodactylum* (Fig 15 and 16). *Phaeodactylum* cell numbers and POC were also linearly correlated (Fig 17).

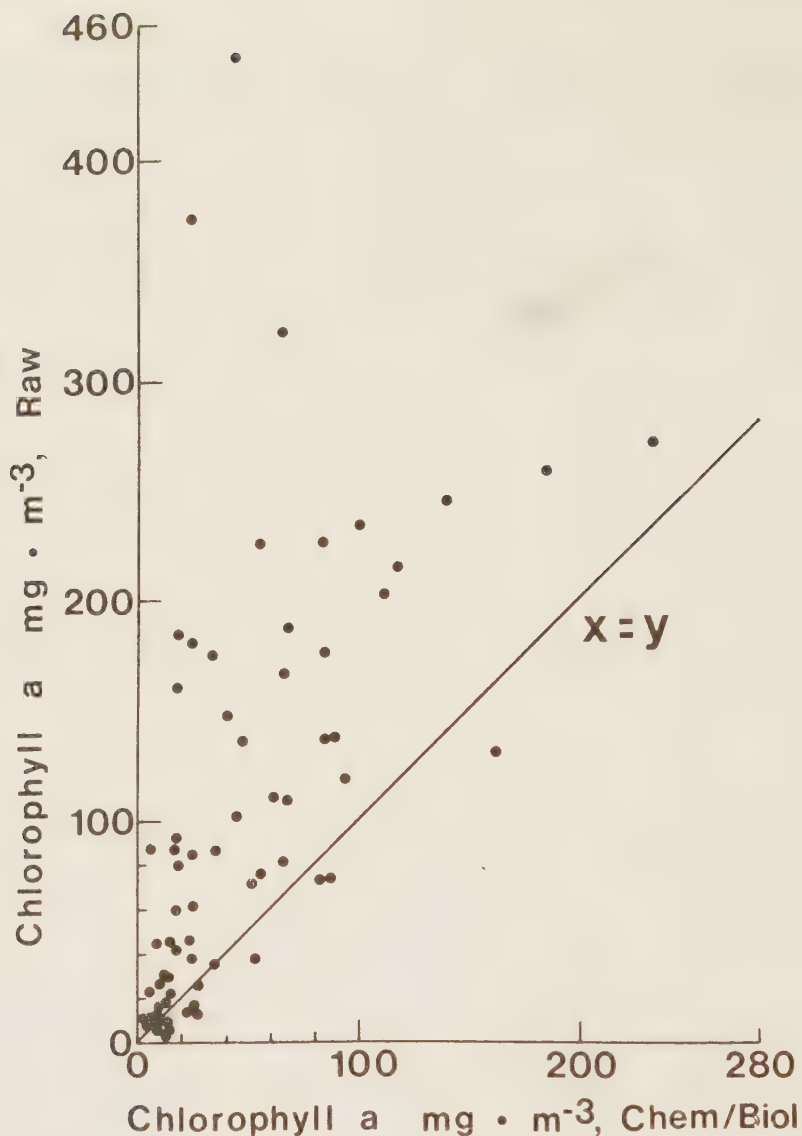


Fig 12. Chlorophyll *a* produced by additions of raw sewage compared to that produced by additions of chemically/biologically treated sewage (both natural communities and *P. tricornutum*)

2. *In situ* experiments

In experiment a (1979-06-16 -- 23) chlorophyll *a* and primary production reached maximal values at approximately the same time, on day 2 for the control, day 4 for addition of chemically-treated sewage, day 5 for 1 % mechanical sewage additions, and days 7 or 8 for 5 % mechanical sewage (Fig 18 and 19). Chlorophyll *a* in the control bag

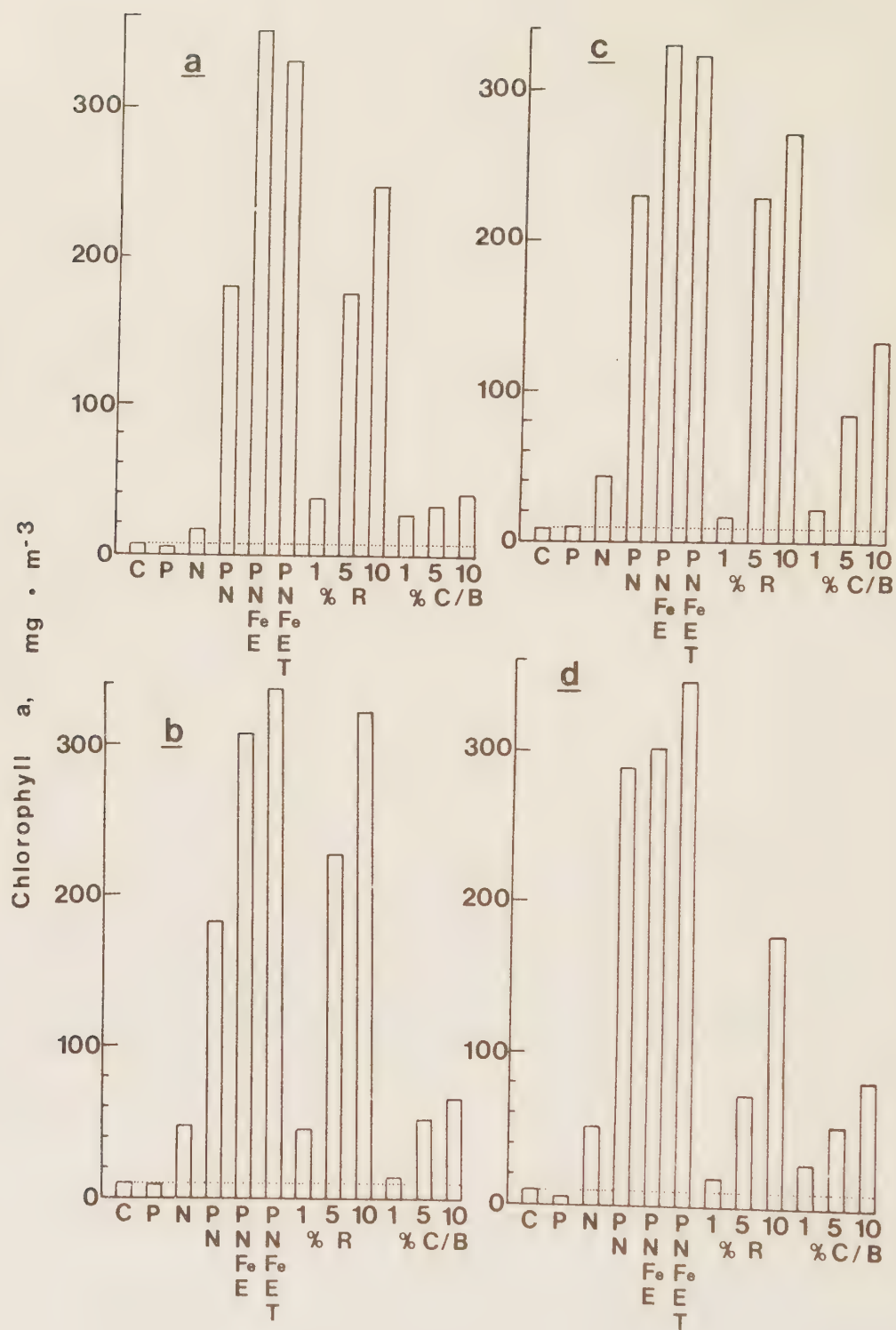


Fig 14. As in Fig 13, but with *P. tricornutum* as the test alga.

increased to slightly above $1 \text{ mg} \cdot \text{m}^{-3}$ with no marked peak, while in the bag with addition of 5 % mechanical sewage from Genarp, a tremendous increase in chlorophyll *a* occurred after the 5th day with a maximum of $77 \text{ mg} \cdot \text{m}^{-3}$ (Tab 3).

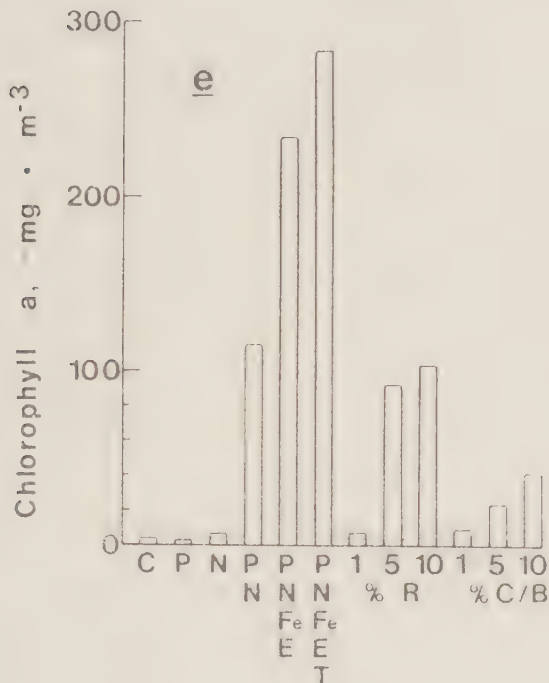


Fig 14.(last part).

The Baltic water used in experiment a had a relatively high inorganic nitrogen (mainly ammonia) content, but a low phosphate concentration (Tab 4). In the mechanically-treated sewage from Genarp ammonia and phosphate concentrations were high, with little nitrate present. In the chemically-treated sewage from the same plant $\text{PO}_4\text{-P}$ was reduced to less than $0.5 \text{ g} \cdot \text{P} \cdot \text{m}^{-3}$ and the N/P quotient was over 50. The mechanically-treated sewage from Källby was similar to mechanical sewage from Genarp, with a low N/P ratio and only small amounts of nitrate. Chemical treatment reduced $\text{PO}_4\text{-P}$ to below $2 \text{ g} \cdot \text{m}^{-3}$, and a large fraction of ammonia was transformed to nitrate. After passage through the oxidation ponds $\text{PO}_4\text{-P}$ was decreased substantially, and the inorganic N/P quotient was over 50.

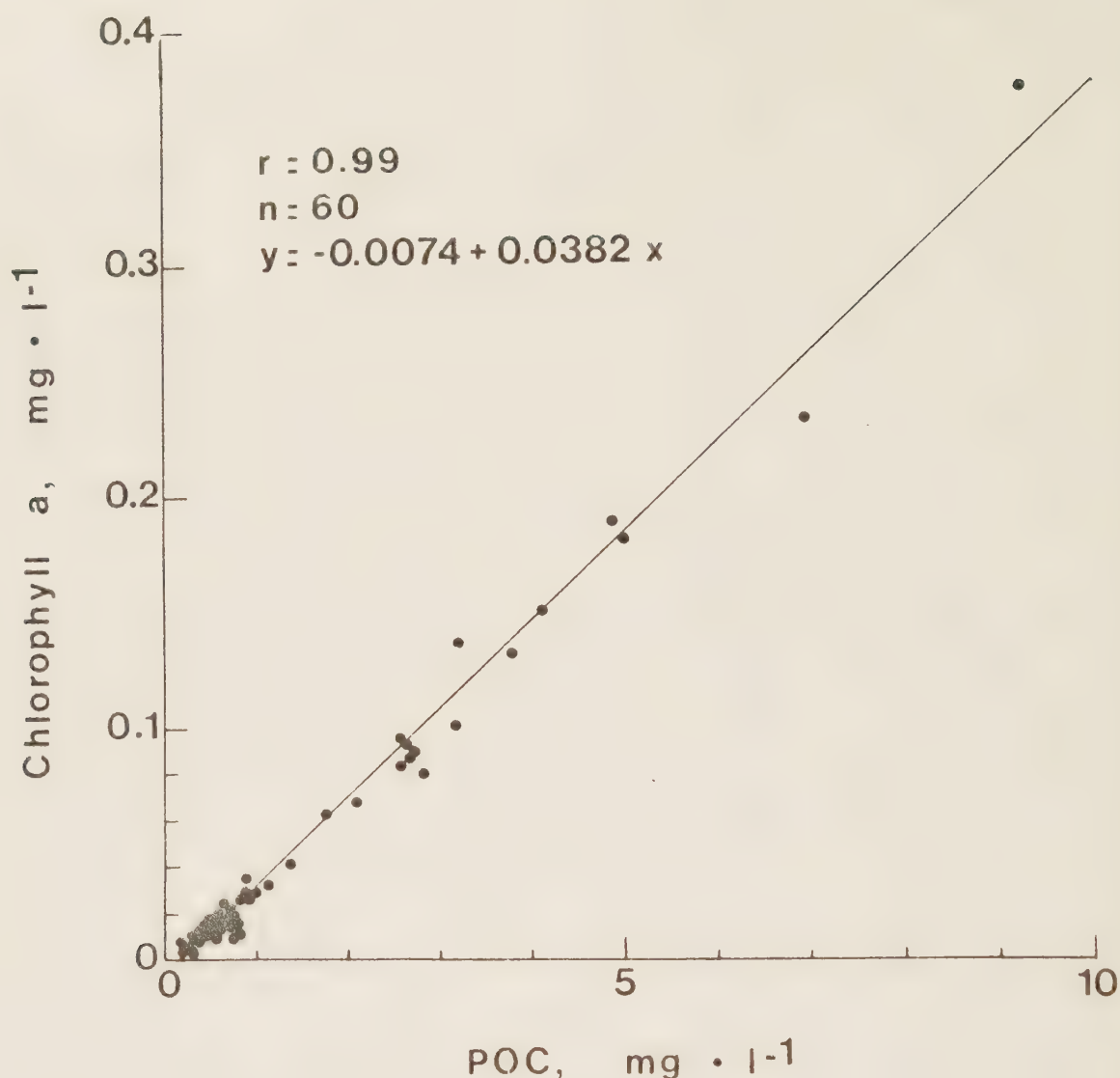


Fig 15. Chlorophyll *a* versus POC (particulate organic carbon) in bottle tests with natural communities. Values from experimental series a-e (cf. Fig 13).

In the control bag ammonia decreased rapidly and was consumed by the 3rd day (Fig 20). In the two bags with mechanically-treated sewage from Genarp ammonia was the dominating inorganic nitrogen fraction. Little inorganic nitrogen was left in the bag with 1 % addition of mechanical sewage at the chlorophyll *a* maximum, while there was still an excess of phosphate. Excess phosphorus was also found

in the 5 % bag at the end of the experiment. Ammonia also dominated over nitrate in the two bags with chemically-treated sewage from Genarp. However, while ammonia was reduced substantially during the experiment, nitrate decreased only slightly (Fig 20). Phosphate was present initially in only low concentrations and was reduced to trace amounts after a few days. In these two bags there were still high concentrations of ammonia at the time of the chlorophyll *a* maxima (Tab 5).

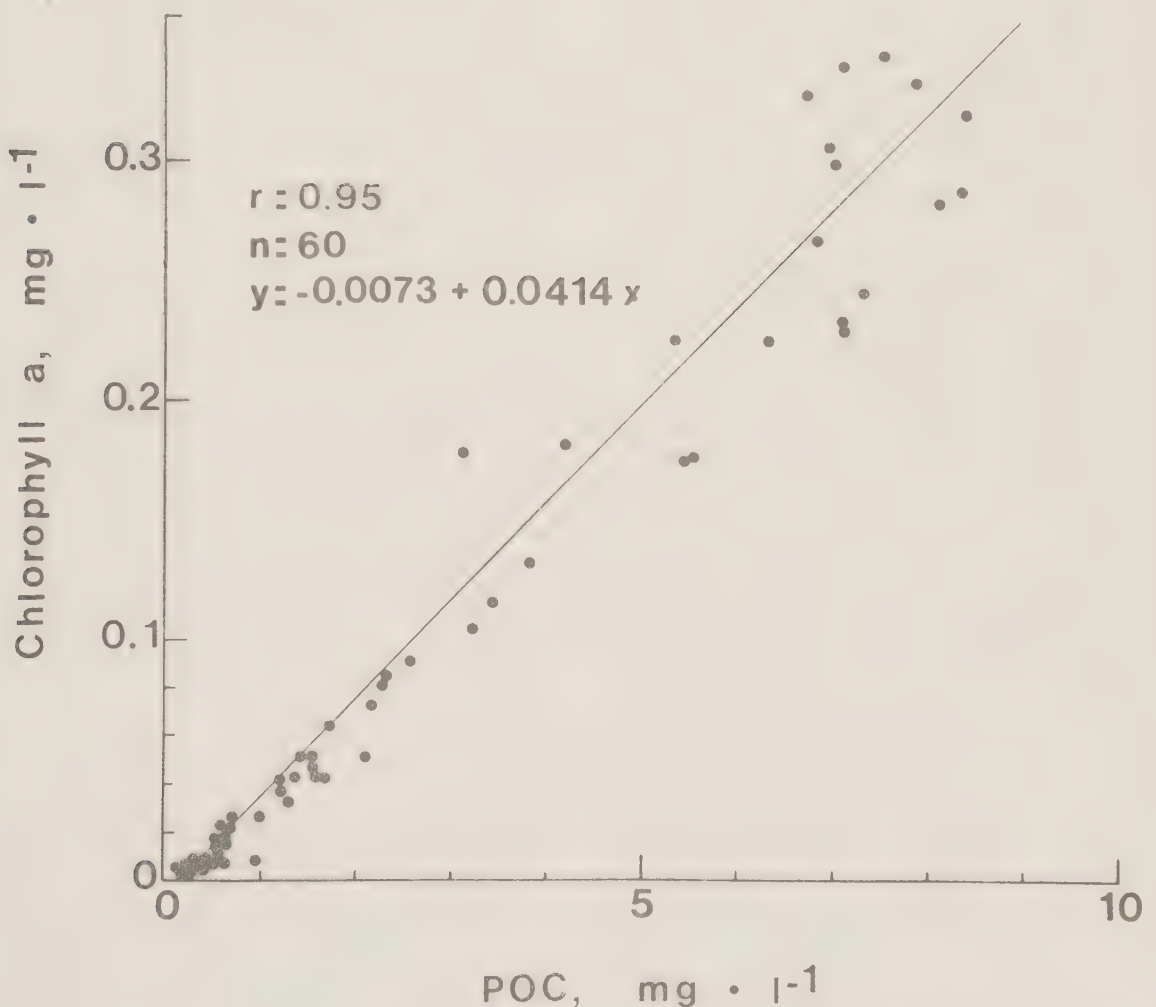


Fig 16. As in Fig 15, but with *P. tricornutum* as the test organism (cf. Fig 14).

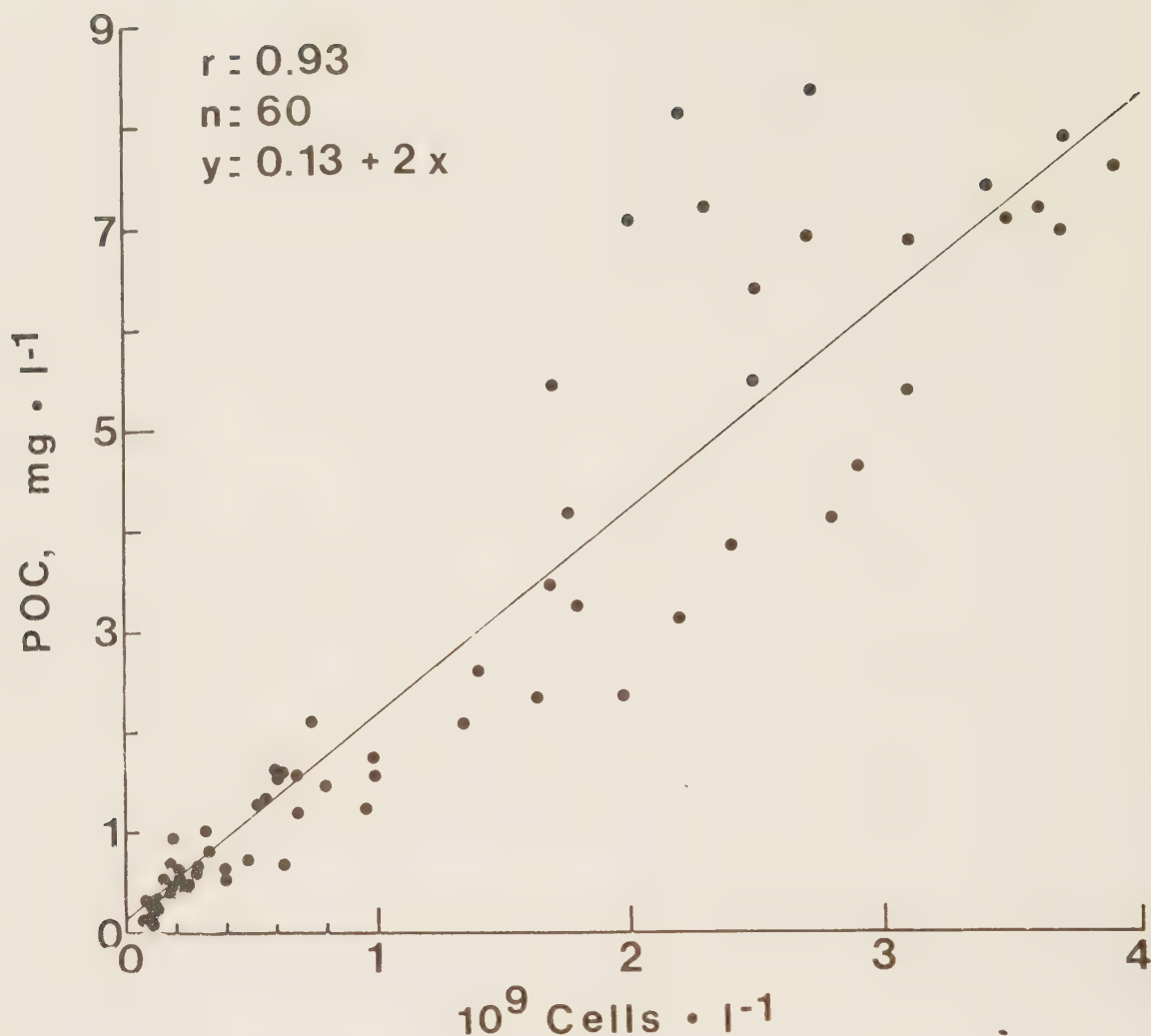


Fig 17. POC versus number of *P. tricornutum* cells.

Changes in ammonia, nitrate and phosphate in bags with mechanically-treated sewage from Källby were very similar to those in corresponding Genarp bags (Fig 21). Phosphate was in excess and inorganic nitrogen absent at the time of the chlorophyll *a* maximum in the bag receiving 1 % Källby mechanical sewage. In contrast to the bags with additions of chemically-treated Genarp sewage, nitrate dominated the inorganic nitrogen in bags with tertiary Källby sewage.

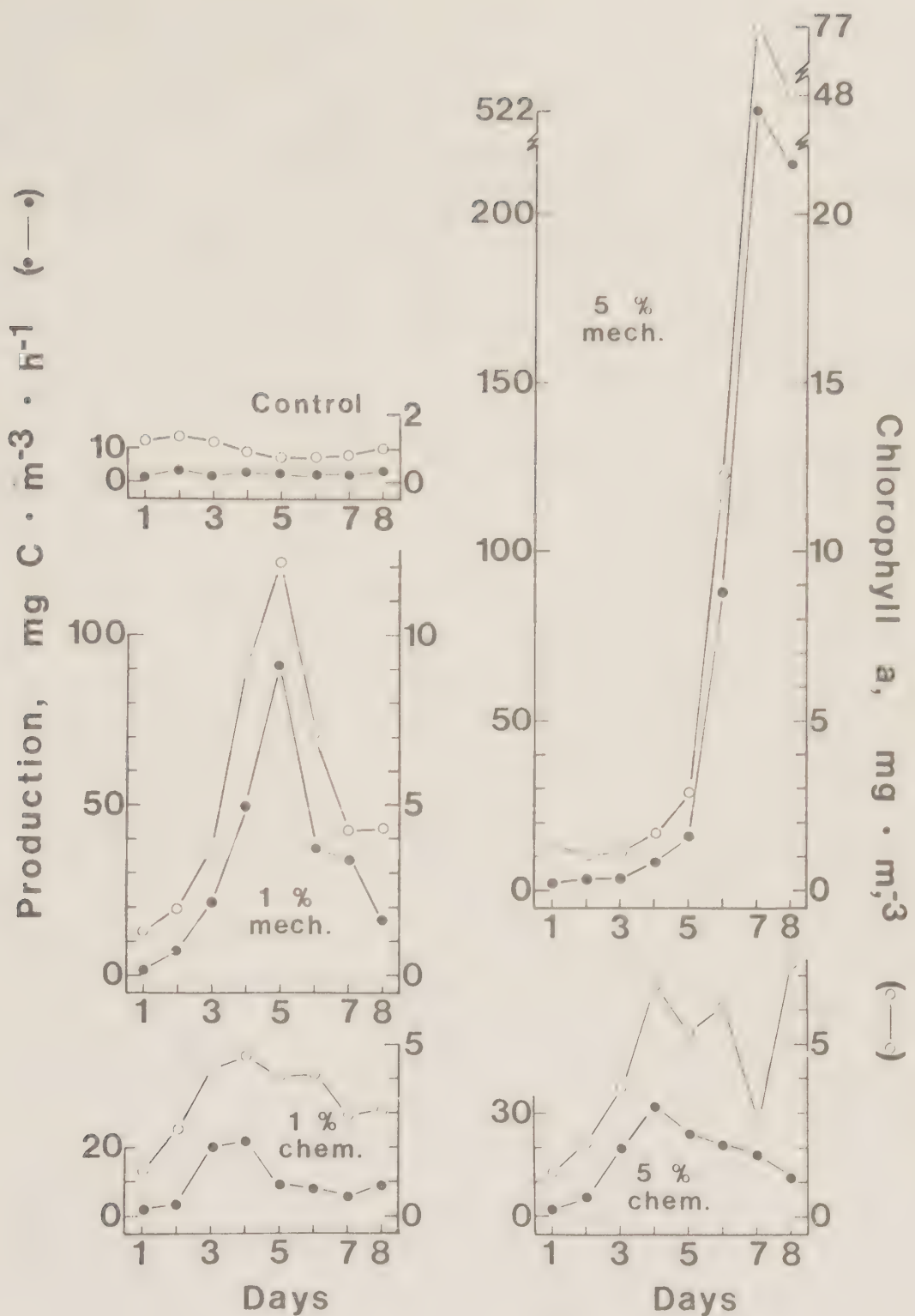


Fig 18. *In situ* experiments (Figs 18 to 23). Exp a= June 16 to 23, 1979. Chlorophyll *a* and ¹⁴C uptake in bags with additions of sewage from Genarp (1 and 5 % of mechanically and chemically treated sewage).

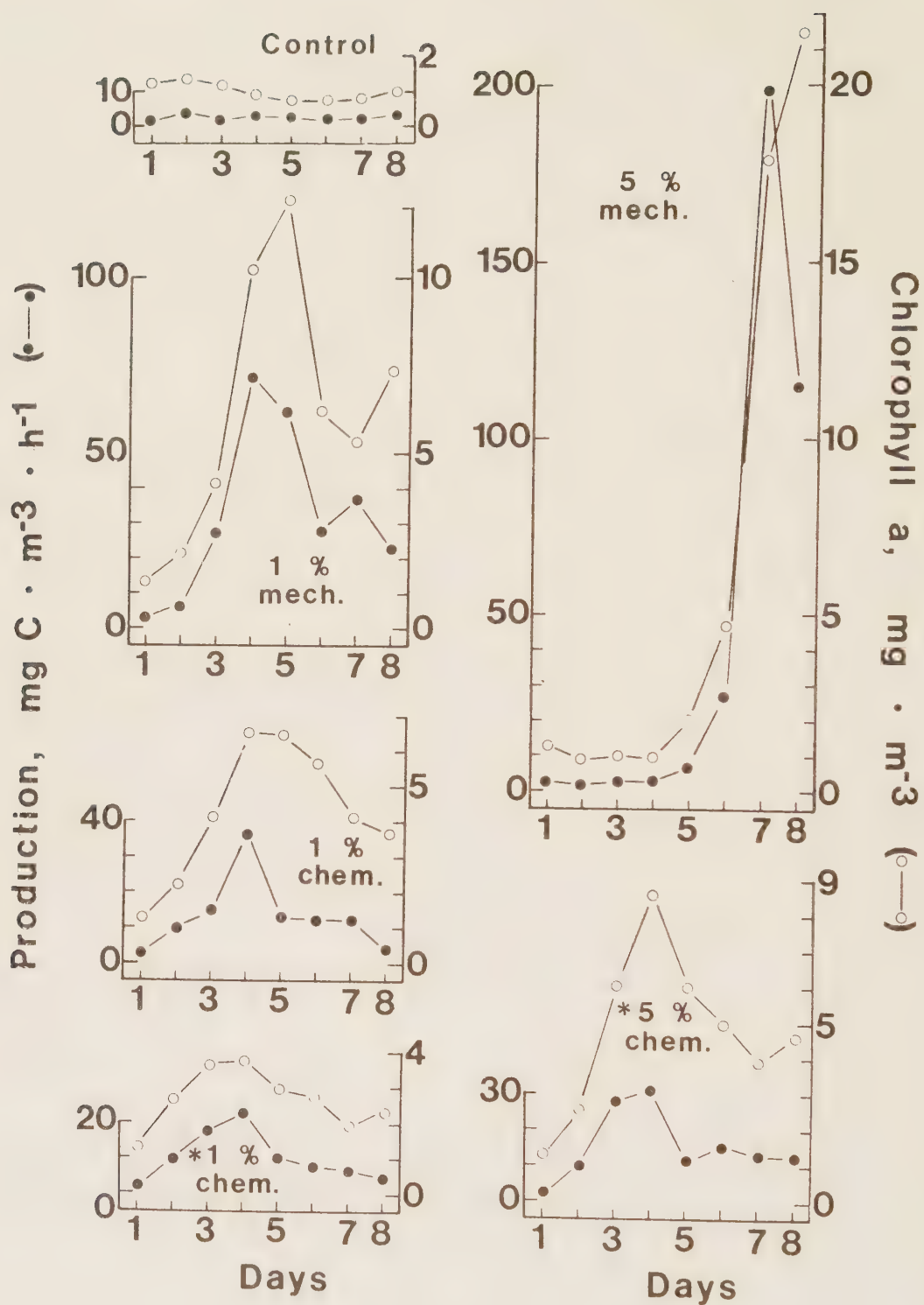


Fig 19. As in Fig 18, but with sewage from Källby. *Chemically treated sewage after passage through oxidation ponds.

Chemical phosphorus removal was less efficient at Källby than at Genarp. However, there was still a large excess of nitrate in the bag with chemically-treated sewage at the chlorophyll α maximum. An even more-pronounced phosphate deficiency was produced in the two bags containing sewage which had passed the oxidation ponds. At the 5 % level high concentrations of nitrate remained even at the termination of the experiment. Ammonia was consumed more rapidly than nitrate in the three bags with chemically-treated Källby sewage.

TAB 3. MAXIMAL CHLOROPHYLL α CONCENTRATIONS IN THE BAGS.

EXP A	CHL α (MG·M ⁻³)
CONTROL	1.40
MECH 1%, GENARP	12.18
" 5%, "	76.85
CHEM 1%, "	4.61
" 5%, "	7.41
MECH 1%, KÄLLBY	12.14
" 5%, "	21.50
CHEM 1%, "	6.43
" 1%, " x)	3.72
" 5%, " x)	8.55
EXP B	
CONTROL	4.52
MECH 1%, KÄLLBY	17.72
BIOL 1%, "	6.07
CHEM 1%, "	10.64

x) AFTER FINAL "OXIDATION" PONDS.

TAB 4. CONCENTRATION OF INORGANIC PHOSPHORUS AND NITROGEN IN THE SEWAGE AND THE WATER USED FOR EXPERIMENTS A (790616 TO 23) AND B (790802 TO 16).

EXP A	PO ₄ -P	NO ₃ -N	NH ₄ -N	NO ₃ -N+ NH ₄ -N	N/P
	/UG·L ⁻¹	/UG·L ⁻¹	/UG·L ⁻¹	/UG·L ⁻¹	
MECH, GENARP	7818	435	31635	32070	4
CHEM, "	410	2600	19206	21806	53
MECH, KÄLLBY	8127	532	29977	30509	4
CHEM, "	1305	17149	3110	20259	16
" , " x)	208	11010	3253	14263	69
WATER	6.2	3.4	40.5	43.9	7
EXP B					
MECH, KÄLLBY	9204	772	26505	27277	3
BIOL, "	3733	19178	617	19795	5
CHEM, "	1233	18655	808	19463	16
WATER	11.5	4.1	6.1	10.1	1

x) AFTER FINAL "OXIDATION" PONDS.

In experiment b (1979-08-02 -- 16) only sewage from the treatment plant in Lund was tested, but both primary, secondary and tertiary sewage at the 1 % level. As in exp a chlorophyll *a* and primary production developed similarly (Fig 22). There was no peak in chlorophyll *a* in the control bag; concentrations increased steadily during the experiment. In the bag with mechanically-treated sewage chlorophyll *a* and primary productivity peaks occurred on day 7, in the bag with biologically-treated sewage on days 5 to 6

and in the bag with chemically-treated sewage on day 6. Tertiary sewage stimulated phytoplankton growth more than secondary sewage (Tab 3). This cannot be explained by initial nutrient concentrations in the bags. Inorganic nitrogen was almost identical in secondary and tertiary sewage, while the latter contained only about one third of the phosphate concentration of secondary sewage (Tab 4).

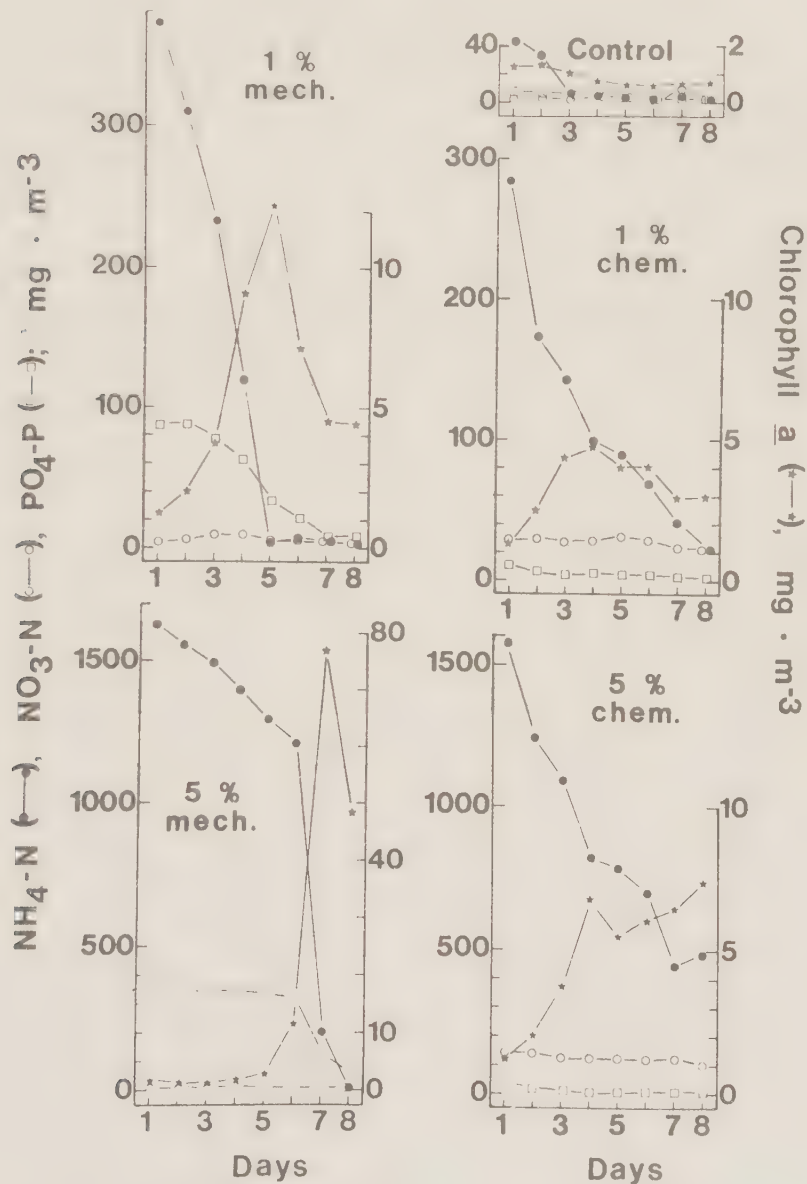


Fig 20. Chlorophyll *a*, nitrogen and phosphorus concentrations in the bags of exp a with Genarp sewage.

The Baltic water used in experiment b had a higher phosphate concentration and much lower inorganic nitrogen concentration than in exp a ($N/P = 0.9$). In the control bag no great changes in concentrations of either phosphate or inorganic nitrogen occurred during the course of the experiment (Fig 23). In the bag with mechanically-treated sewage some phosphate, but little inorganic nitrogen, remained at the time of the chlorophyll α peak and onwards (Tab 5). In the other two bags both phosphate and inorganic nitrogen were utilized efficiently. Ammonia was preferentially taken up compared to nitrate in the bag with mechanically-treated sewage.

TAB 5. (CHLOROPHYLL α PRODUCED (MAXIMAL VALUE-INITIAL VALUE), NUTRIENTS CONSUMED (INITIAL VALUE-VALUE AT CHL α MAXIMUM) AND NUTRIENTS LEFT IN THE WATER AT THE CHL α MAXIMUM FOR THE DIFFERENT BAG EXPERIMENTS.

EXPERIMENT A	CHLOROPHYLL α MG·M ⁻³	PO ₄ -P MG·M ⁻³		NH ₄ -N MG·M ⁻³		NO ₃ -N MG·M ⁻³	
	PRODUCED	CONSUMED	LEFT	CONSUMED	LEFT	CONSUMED	LEFT
CONTROL	0.12	0.31	5.91	8.5	32	0.53	2.87
CHEM. 1 %, GENARP	3.33	3.73	6.22	185.59	98.41	0.55	28.14
" 5 %, GENARP	5.42	20.51	8.08	750	821	16.7	125
" 1 %, KÄLLBY	5.15	15.72	7.46	178.11	4.89	153.99	42.01
" " " X)	2.44	1.87	5.28	65.76	4.54	111.82	35.18
" 5 %, " X)	7.27	8.70	8.70	206.28	7.52	27	512.3
MECH. 1 %, GENARP	10.9	54.08	33.57	367.98	4.02	-1.02	4.71
" 5 %, "	75.57	236	124.01	1429	202	-5.94	11.61
" 1 %, KÄLLBY	10.86	60.61	27.97	333.68	3.32	-0.07	3.96
" 5 %, "	20.22	380.11	27.01	1649.88	6.12	-0.75	6.69
EXPERIMENT B							
CONTROL	3.66	4.01	7.46	2.10	4.02	0.91	3.10
CHEM. 1 %, KÄLLBY	9.78	16.03	7.78	10.26	4	202.86	3.14
BIOL. " " "	5.21	35.80	14.30	9.21	3.15	191.90	4.10
MECH. " " "	16.86	79.74	24.86	277.85	3.15	9.72	2.53

X) AFTER FINAL "OXIDATION" PONDS.

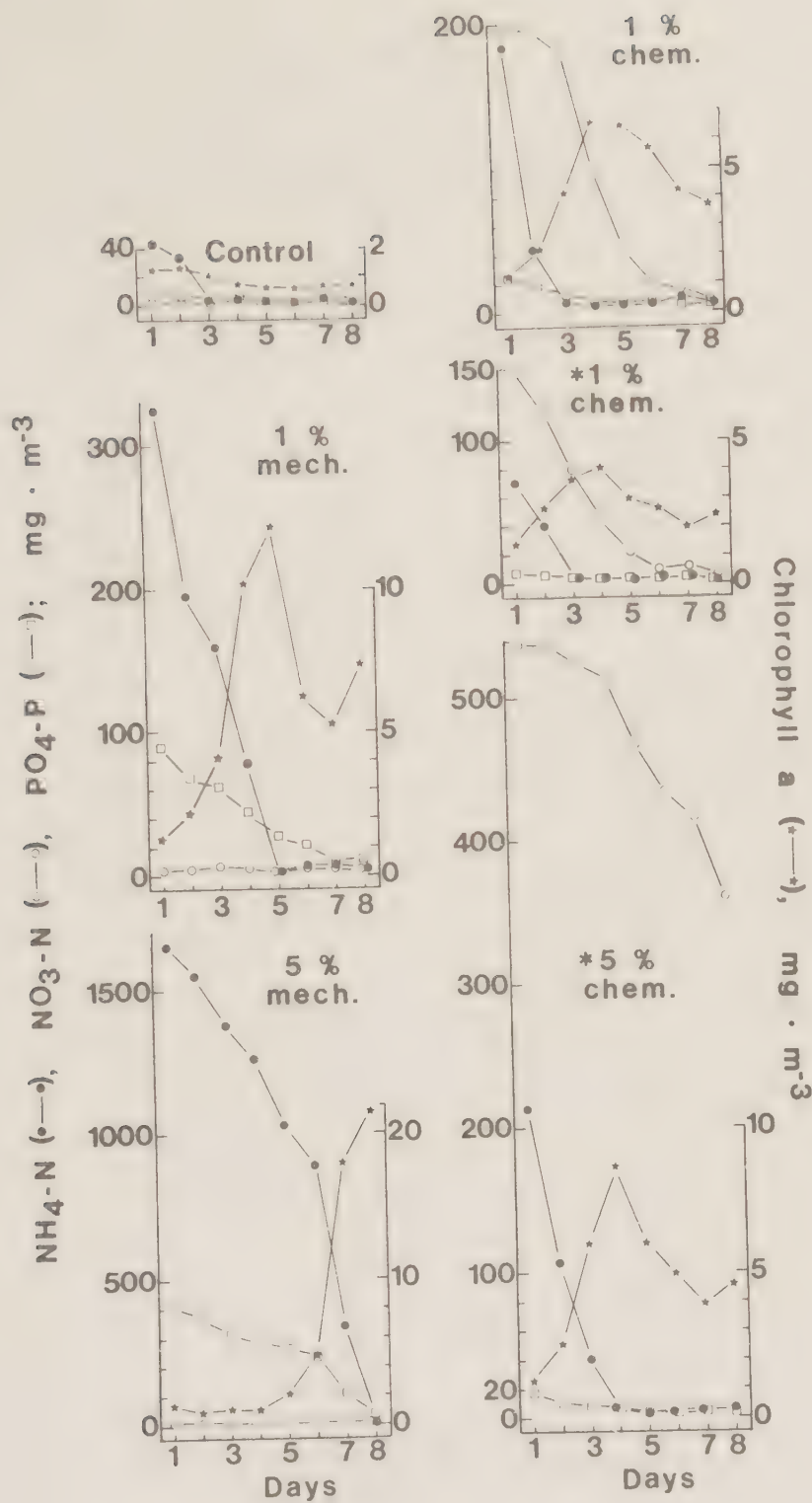


Fig 21. Chlorophyll *a*, nitrogen and phosphorus concentrations in the bags of exp a with Källby sewage.
 * Chemically treated sewage after passage through oxidation ponds.

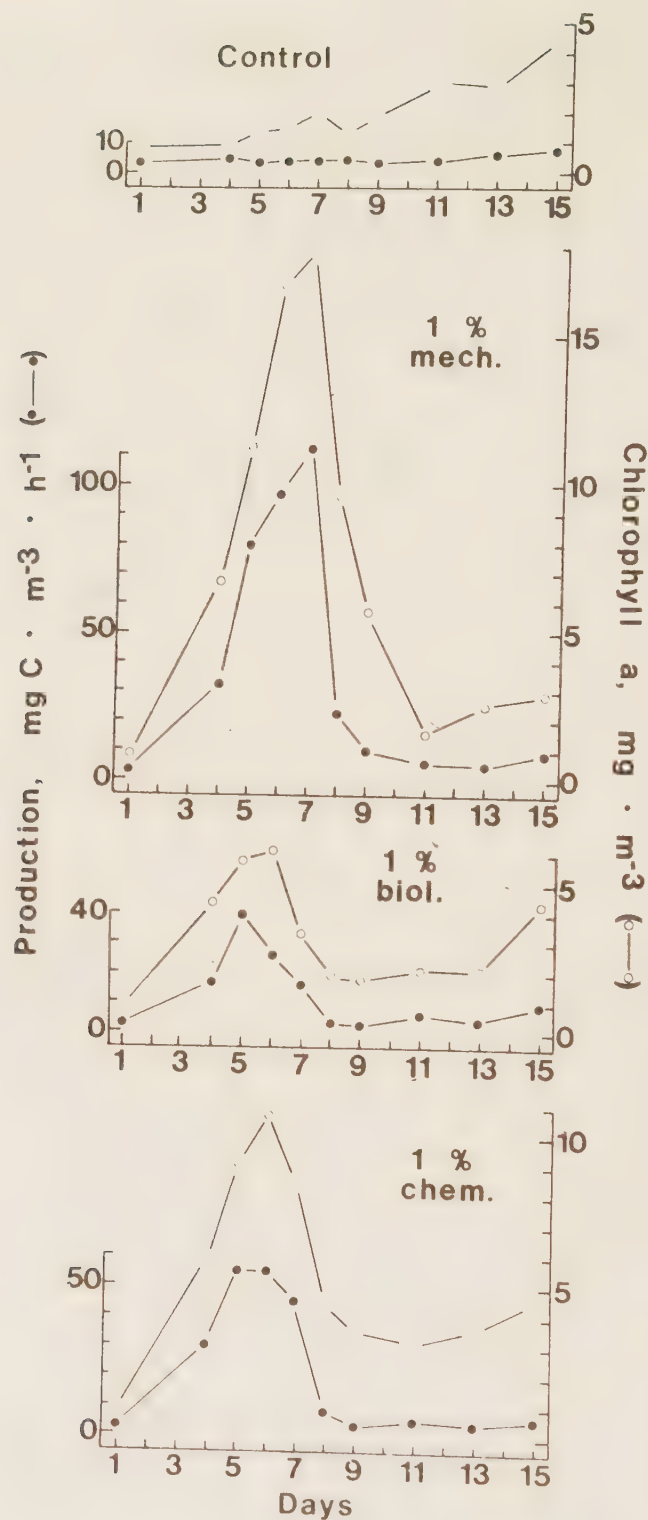


Fig 22. Exp b (August 2 to 16, 1979). Chlorophyll *a* and ¹⁴C uptake in bags with additions of 1 % mechanically, biologically or chemically treated sewage from Källby.

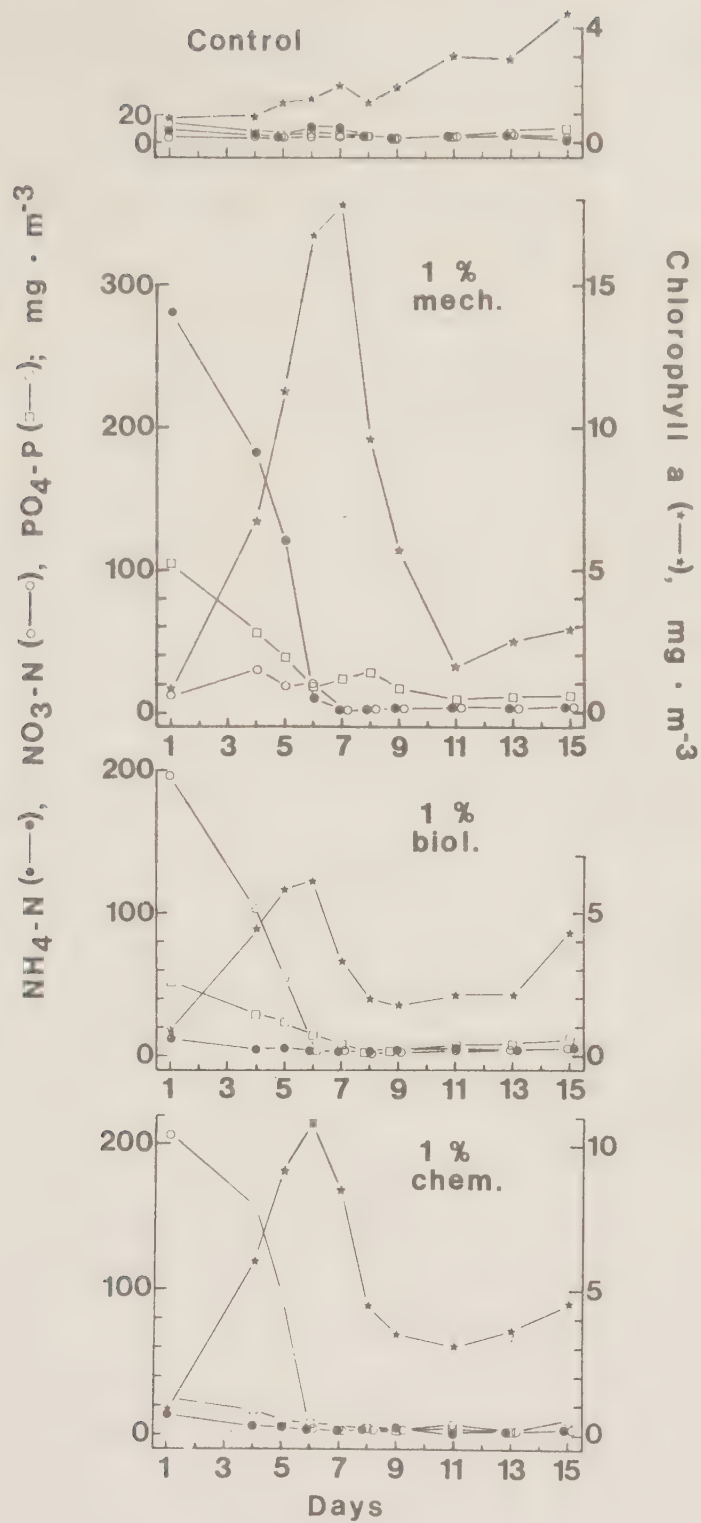


Fig 23. Exp b. Chlorophyll *a*, nitrogen and phosphorus concentrations in bags with 1 % mechanically, biologically or chemically treated sewage from Källby.

Quotients between inorganic nitrogen and phosphate consumed from the start of the experiment until the chlorophyll a maxima were high (>10) for bags with chemically-treated sewage but low (<10) for bags with biologically and mechanically-treated (Tab 6). Ratios between nutrients consumed and amount of chlorophyll a produced were highly variable without systematic changes (Tab 6). As nutrient uptake continued even

TAB 6. QUOTIENTS BETWEEN CHLOROPHYLL a PRODUCED AND NITROGEN AND PHOSPHORUS CONSUMED IN EXPERIMENTS A AND B IN THE FALSTERBO CHANNEL.

	N/P	N/CHL a	P/CHL a
EXPERIMENT A			
CONTROL	29.13	75.25	2.58
CHEM. 1 %, GENARP	49.90	49.90	1.12
" 5 %, "	37.38	141.46	3.78
" 1 %, KÄLLBY	21.18	65.66	3.05
" " , " X)	97.96	72.78	0.77
" 5 %, " X)	26.81	32.09	1.20
MECH. 1 %, GENARP	6.79	33.67	4.96
" 5 %, "	6.03	18.83	3.12
" 1 %, KÄLLBY	5.50	30.72	5.58
" 5 %, "	4.34	81.55	18.80
EXPERIMENT B			
CONTROL	0.77	0.85	1.10
CHEM. 1 %, KÄLLBY	13.30	21.79	1.64
BIOL. " " "	5.73	38.60	6.73
MECH. " " "	3.61	17.06	4.73

X) AFTER FINAL "OXIDATION" PONDS.

after chlorophyll *a* concentrations started to decrease, nutrient uptake and chlorophyll *a* production were partly uncoupled processes.

DISCUSSION

Measures of response of algal populations to nutrient enrichment

Algal growth is discussed here mainly in terms of chlorophyll *a* and inorganic carbon uptake. ^{14}C fixation is - if light, temperature, nutrients etc. are not limiting - a function of chlorophyll *a* concentration. Therefore, these two parameters should be roughly proportional, which was the case for *in situ* experiments. The influence of nutrient enrichment on assimilation numbers ($\text{mg C}_{\text{fixed}} \cdot \text{mg chl } a^{-1} \cdot \text{h}^{-1}$) will be discussed later.

Chlorophyll *a* is not proportional to phytoplankton biomass (biovolume). It has been shown that the amount of chlorophyll *a* in an algal cell is a function of e.g. light (Beale and Appleman 1971), growth rate (Caperon and Meyer 1972, Thomas and Dodson 1972, Eppley and Renger 1974) and nutrient limitation (Laws and Bannister 1980). Light can probably be ruled out as a factor in the laboratory experiments since it was continuous and, at least externally on the bottles, the same for all tests. Furthermore, growth rate was approximately zero since the algae had reached the stationary phase by the tenth day, when chlorophyll *a* was measured (cf. Leonardson 1971). Nutrient availability was probably variable, because of the different amounts of sewage added. Nevertheless, POC and chlorophyll *a* were linearly correlated both for *Phaeodactylum* (Fig 16) and the natural populations (Fig 15), with C/chl *a* quotients of 24 and 26, respectively. These values are typical for phytoplankton which are not nutrient limited (Granéli 1981, de Jonge 1980).

In the bag experiments chlorophyll *a* was the only estimate of phytoplankton biomass used. In earlier *in situ* experiments in the Falsterbo Channel it was found that at high chlorophyll *a* concentrations - following additions of N and P - the C/chl *a* ratio tended to decrease (Granéli in press). Thus it might be concluded that for the laboratory bioassays, chlorophyll *a* did measure phytoplankton biomass, but for the *in situ* bag experiments high chlorophyll *a* values gave a false impression of biomass increase. From a water quality point of view this discrepancy may not be important, as transparency, which is a prime measure of recreational water quality, is a function of chlorophyll (Forsberg and Ryding 1980).

Algal stimulation and inhibition in sewage water

Biologically-treated (secondary) sewage is an excellent enrichment medium for algal cultures (Ansell et al. 1963, Goldman 1976). Dunstan and Menzel (1971) were able to maintain mixed populations of marine phytoplankton in continuous cultures with up to 20 % secondary sewage at dilution rates of 0.5 per day. Goldman and Stanley (1974) successfully grew several species of phytoplankton in continuous cultures with 50 % sewage/seawater mixtures. In experiments with sewage from Källby mixed with Öresund water (Salviken at Barsebäck) Leonardson (1971) grew *Phaeodactylum tricornutum* in 75 % biologically-treated sewage.

The sewage (raw, mechanical, biological or chemical and from three different treatment plants) used in the present investigation increased chlorophyll *a* synthesis in almost all mixtures of up to 10 % in Öresund water. Growth in terms of chlorophyll *a* was lower in 1 % raw or tertiary Sjölanda sewage than in controls with only Öresund water on a few occasions. This cannot be interpreted as a sign of growth-inhibiting substances, however, as algae grown in 5 and 10 % sewage always showed higher chlorophyll *a* values than

control bottles. A large treatment plant such as Sjölanda receives sewage from many industries discharging toxic substances. Although such substances, e.g. toxic metals, were present, they were obviously dilute enough not to stop algal growth.

Inhibition, indicating the presence of toxic substances, has been reported in AGP (Algal Growth Potential) tests with sewage from treatment plants in Sweden (see e.g. Forsberg et al. 1978b) and in receiving waters (Thomas et al. 1974). The inhibitory effect is probably dependent on the test algae used. In my bottle experiments *Phaeodactylum tri-cornutum* usually grew better than natural phytoplankton communities from the Öresund.

In the *in situ* experiments biologically-treated sewage was tested only in experiment b. The maximal chlorophyll *a* concentration was only one third of that in mechanically-treated sewage, and even lower than in tertiary sewage (Tab 3). One can suspect inhibition in the bag with secondary sewage, as the phosphorus and nitrogen content would imply a higher chlorophyll *a* value. In experiment a phytoplankton growth was relatively good in tertiary sewage from Källby when compared to Genarp tertiary sewage. Concentrations of inorganic nitrogen were more or less equal, while tertiary sewage from Genarp contained only about one-third of the phosphate found in Källby sewage (taken before passage through the final ponds).

In addition to differences in phosphate content, another factor may be involved in these differences in growth-stimulating effects. In Genarp phosphorus is precipitated with aluminium sulphate and in Källby with iron chloride. Aluminium can inhibit algal growth, e.g. if there is an overdosage in the treatment plant (Forsberg and Hökervall 1972). Iron, on the other hand, can be highly stimulatory

for marine phytoplankters, especially in oligotrophic areas (Menzel et al. 1963). In combination with organic chelators the stimulating effect can be strengthened (Smayda 1974). In the nutrient enrichment tests made parallel to the sewage bioassays, addition of a combination of EDTA and iron had a stimulatory effect on several occasions (Fig 13-14).

N/P quotients, limiting nutrients and effects of sewage on the Öresund

The average inorganic N/P ratio for raw sewage from Sjölundå was 6.0 which is in the range supposed to be optimal for phytoplankton growth (Chiaudani and Vighi 1974). Forsberg et al. 1978b) suggested that N/P ratios in lake water between 5 and 12 indicate that either N or P or both nutrients may act as growth-limiting substances. Ryther and Dunstan (1971) compared N/P ratios in sewage, coastal waters and marine phytoplankton, and concluded that the latter have an optimum around 4.5. In laboratory cultures phytoplankton may vary enormously in chemical composition and still appear "healthy". However, a good agreement between evaluations of the most limiting nutrient based on N/P ratios in the water and that based on enrichment tests has been found (Forsberg et al. 1978, Rinne and Tarkkainen 1978). This indicates that phytoplankton under natural conditions maintain a more or less constant cellular composition.

Chemically-biologically treated sewage from the Sjölundå plant had a higher mean N/P quotient than the optimum for phytoplankton. The value (18.9) is in a range where phosphorus should be limiting. Inorganic nitrogen concentrations were similar in raw and tertiary sewage from Sjölundå. Thus raw sewage mixed with Öresund water should produce more phytoplankton biomass (chlorophyll *a*) than equivalent mixtures with tertiary sewage, and this was the case. The mean quo-

tient between phosphate concentrations in raw and tertiary sewage was 3.2 for Sjölanda. Average quotients between chlorophyll *a* produced in bottles with 1 % raw sewage and 1 % tertiary sewage were 1.5 and 1.3 for natural populations and *Phaeodactylum*, respectively. Individual variations were large, so these mean values should not be greatly emphasized (cf. Tab 1 and 2). The values indicate, however, that phytoplankton biomass in receiving waters would not be reduced in proportion to phosphorus removal. This effect should be even more pronounced in the real situation, as sewage addition is less than 1 %. The explanation is the low N/P quotient in Öresund water. In contrast to the chemically/biologically-treated sewage, phosphorus is found in excess in the Öresund in comparison to the needs of algae.

It should be noted that the bottle bioassays did not indicate only treatment efficiency, but also the direct effect of sewage on phytoplankton in the recipient. Forsberg (1972) and Claesson and Forsberg (1978) used "standard recipients" to test the quality of sewage and found that phosphorus concentrations and algal volumes were proportional (Forsberg and Forsberg 1972, Forsberg et al. 1978b).

Although sewage treatment has focused on phosphorus elimination, nitrogen seems to be a more crucial element for phytoplankton in the Öresund. Nitrogen limitation has been described for a number of oceanic and coastal waters and seems to be a general phenomenon (Thomas 1969, 1970, Thomas et al. 1974, Ryther and Dunstan 1971, Vince and Valiela 1973, Doig and Martin 1974, Smayda 1974, Goldman 1976, Lännergren 1978), while records of phosphorus limitation in marine environments are more limited (Berland et al. 1978).

In the Baltic proper nitrogen has been indicated as the most likely limiting nutrient for phytoplankton production

(Sen Gupta 1972). Additions of nitrogen alone to Öresund water usually stimulate phytoplankton growth considerably, while phosphate additions do not have this effect (Granéli 1981, Sundström et al. 1980). Chlorophyll concentrations tripled (annual mean) after enrichment with nitrate, while phosphate additions did not increase chlorophyll *a* (Granéli 1980). With respect to phytoplankton, it seems clear that nitrogen is the primary limiting nutrient *sensu* Liebig in the Öresund, as in many other coastal areas.

Sewage treatment policies

The only effect of sewage on the recipient to be discussed here is eutrophication, i.e. increasing nutrient availability causing increased primary production. Of course, sewage treatment policy is not solely based on this criterium. Other aspects may be as important, e.g. hygienic standards (coliforms), oxygen consumption by primary BOD, elimination of toxic elements, etc. Chemical precipitation removes not only phosphorus, but also part of the heavy metals as well as BOD. However, the decision to introduce tertiary treatment of municipal sewage in Sweden must have been directed almost exclusively towards lessening cultural eutrophication (Svensson 1971). Generalizations concerning effects of removal of phosphorus from sewage discharged to lakes are probably warranted (Schindler 1974), as phosphorus seems to be growth limiting for phytoplankton in many unpolluted lakes (Parr and Smith 1976, Welch et al. 1978). To extend this generalization to Sweden's coastal recipients seems scientifically dangerous (cf. Hannerz 1971).

It has been shown that nitrogen removal from municipal sewage entering coastal waters eliminates eutrophicating effects almost entirely (Goldman et al. 1973, Goldman 1974a and b). It has also been suggested that removal of deter-

gent phosphorus in communities with a marine sewage outfall would not affect eutrophication (Goldman 1976, Doig and Martin 1974).

Although a large fraction of Swedish municipalities already have tertiary sewage treatment facilities (phosphorus removal), and still more tertiary treatment plants are planned or under construction, debate concerning the usefulness of this sewage treatment for coastal municipalities has not stopped (Hallqvist 1980, Söderström 1980). A common argument in favour of phosphorus removal is that phosphorus does not presently limit production in our coastal recipients because they have been fertilized with secondary sewage containing an excess of phosphorus. In polluted lakes nitrogen often becomes limiting for phytoplankton production (Claesson and Ryding 1975). Efficient removal of phosphorus would once again make it the limiting nutrient, as was the case before pollution started.

This situation is hardly valid for the Öresund. Nutrient concentrations in the Öresund are still relatively low (Dahl-Madsen 1980) compared to polluted lakes. Water quality in the Öresund is determined not only by the large discharge of sewage, but also by the enormous inflow of unpolluted Baltic and Kattegat water. Limiting nutrient conditions in the Öresund probably reflect natural conditions and not nutrient proportions in sewage (Granéli 1981). This distinction is important, as has been pointed out by Caperon et al. (1971). In the polluted Lomma bay, which receives tertiary sewage from Sjölanda, phosphorus has been found to be in deficit in comparison to nitrogen (Granéli and Sundbäck unpublished).

Growth stimulating effects of municipal sewage have mainly been discussed here in terms of nitrogen and phosphorus. Although final yield in algal assays with sewage additions

is often in proportion to the amount of nitrogen or phosphorus added (Doig and Martin 1974, Goldman et al. 1974 b), other substances may be of importance. In the bottle tests N and P added in raw sewage could produce higher chlorophyll *a* concentrations than when larger amounts of pure nutrient solutions were added. As the additional growth-regulating factors have not been identified and the technical and economical feasibility of controlling them is not known, sewage treatment will only be discussed in terms of phosphorus and nitrogen.

Effects of nutrient enrichment on phytoplankton

Before present and future sewage treatment in the Öresund region is discussed in more detail, the relevance of sewage and nutrient enrichment experiments for the discussion must be clarified. Limiting nutrients can be considered to act on microscopic algae in two ways (O'Brien 1972, Rodhe 1978). First, a nutrient in short supply relative to other nutrients will limit the final yield (cell number, biovolume, cell carbon, chlorophyll etc.) in batch-type situations. Secondly, the growth rate (e.g. doublings per day) of a phytoplankton population can be controlled by the external and/or internal concentration of a nutrient (Droop 1973). The relation between nutrient concentration and growth rate has often been described by the Michaelis-Menten hyperbolic equations (Caperon and Meyer 1972). The availability of a nutrient for phytoplankton growth is not determined only by external sources (upwelling, atmosphere, discharge), but also by regeneration in the immediate surroundings of the cell (autolysis, bacterial activity, grazing) (Dugdale 1967). Increased growth rate may not be associated with increased biomass, as the death rate (through senescence, grazing, parasitism and sedimentation) may increase more or less in proportion to growth rate. In this way a steady state with respect to nutrient concentrations

and biomass can be achieved, as is found in chemostats and which may be approached in tropical seas (Eppley et al. 1973). In temperate waters batch-type phenomena are perhaps more common, due to fluctuating environmental conditions (light, temperature). An example is the spring outburst of diatoms after the build-up of nutrients during winter.

In this article the relation between nutrient concentration and phytoplankton is discussed in terms of potential biomass yield, i. e. the "Liebig" concept of limiting nutrients. For water quality criteria biomass is of greater interest than growth rate. With respect to secondary production (e. g. commercially valuable fish species) biomass turnover may be more relevant.

Biomass yield, as found in an enrichment experiment, is only a potential maximum that may not be reached in the real situation. Increased nutrient concentrations may have an immediate effect on the photosynthetic capacity of algae, however, and this was found in the sewage enrichment experiments. Assimilation numbers increased compared to the values in the control bags in both exp a and b (Fig 24 and 25). The effect was seen for mechanical as well as chemical sewage. Increased assimilation numbers after enrichment with phosphate, ammonia or nitrate were found in bag experiments in the Falsterbo Channel (Granéli 1981). These assimilation numbers were not necessarily $P_{\max}$, however, as photosynthesis was not measured in relation to radiation. The absolute values should therefore be used with caution.

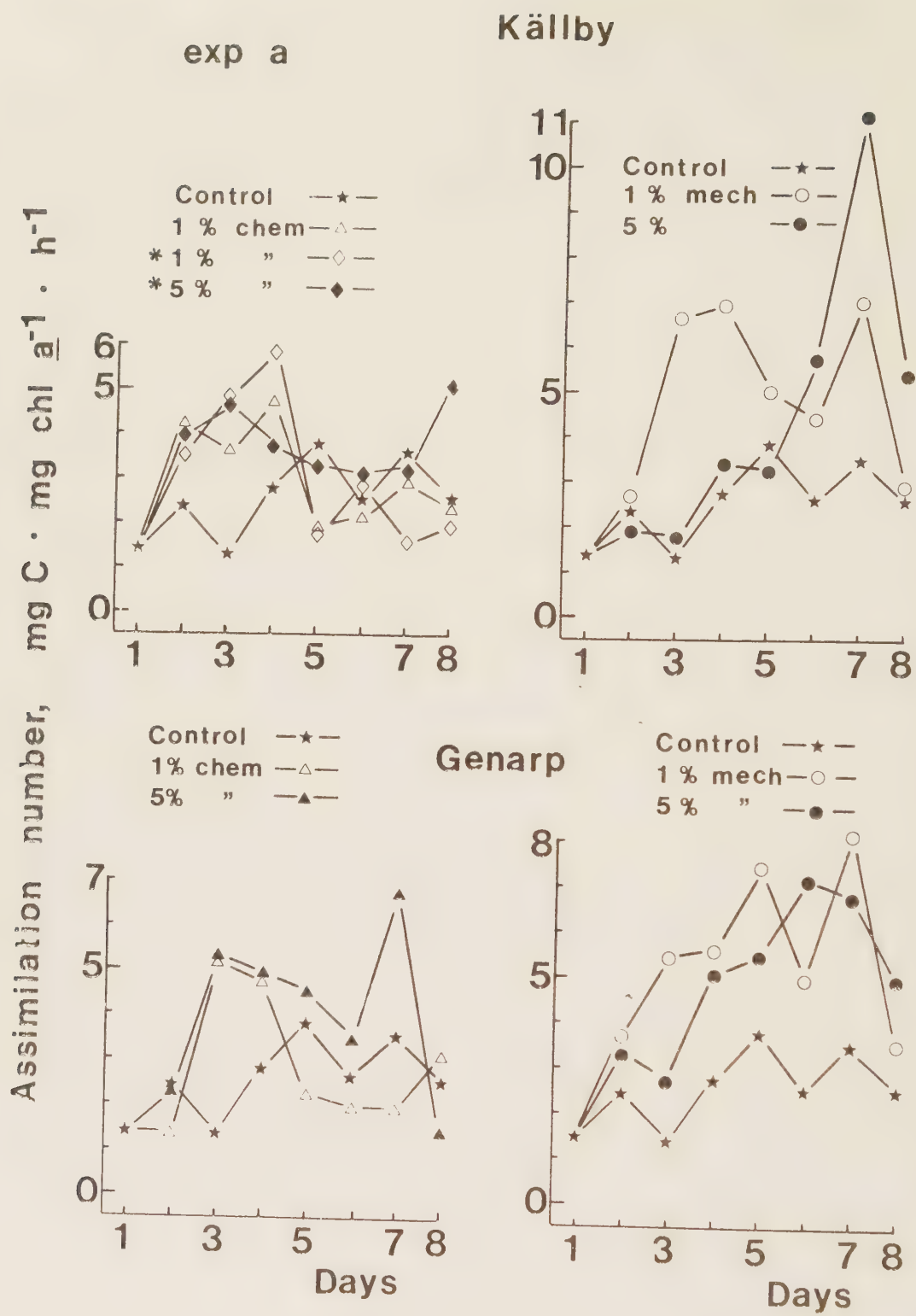


Fig 24. Assimilation numbers in the bags of exp a.

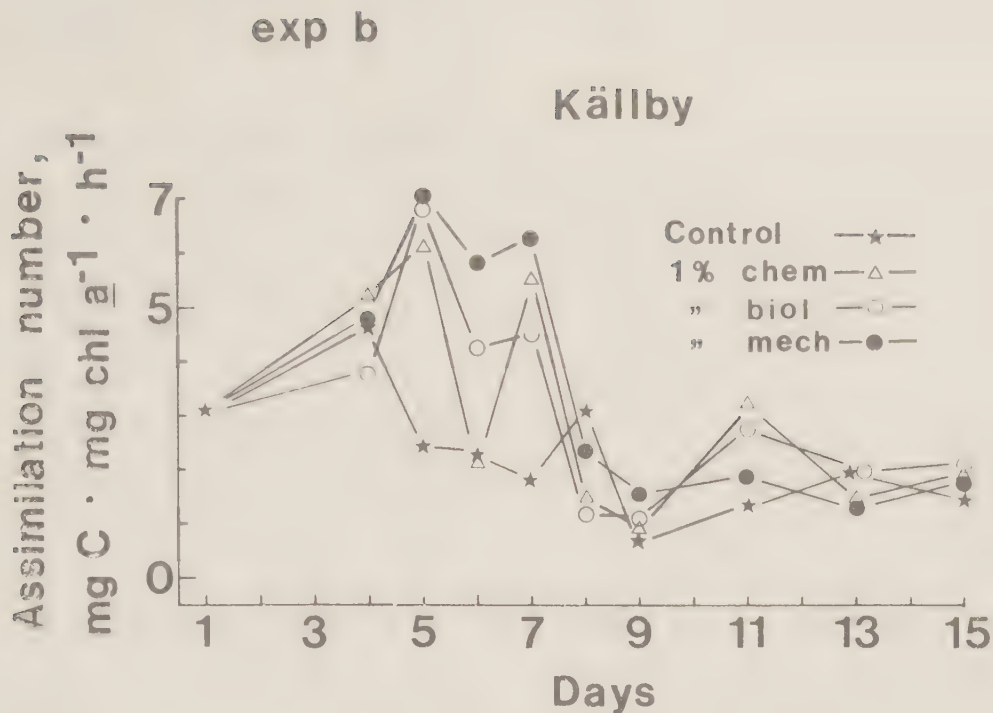


Fig 25. Assimilation numbers in the bags of exp b.

Nutrients in sewage, phytoplankton and Öresund water

It is at present not economically feasible to remove both phosphorus and nitrogen from sewage (which would give the most efficient reduction in eutrophicating effect). Relative proportions of N and P in sewage, in the recipient and in algal cells provide a starting point in answering the question of what should be removed.

Both primary and secondary sewage have low N/P ratios, in Sweden usually between 3 and 6 (Ulmgren 1975, Elfving et al. 1975). In USA lower ratios have been reported, probably due to higher phosphorus concentrations (Dunstan and Menzel 1971, Goldman and Stanley 1974, Goldman 1976). Minor variations are found depending on whether ratios are calculated with inorganic fractions (which make up

the bulk of total N and P in raw, primary and secondary sewage), or total N and P.

N/P quotients of chemically-treated sewage should be quite high. Swedish tertiary treatment plants are usually built to achieve 90 % P removal and give final effluent concentrations around $0.5 \text{ g tot-P} \cdot \text{m}^{-3}$. As outgoing water has a tot-N concentration around $20 \text{ g} \cdot \text{m}^{-3}$, N/P quotients in tertiary sewage should be around 40. Elfving et al. (1975) reported annual mean $\text{PO}_4\text{-P}$ and tot-P concentrations for 5 tertiary treatment plants varying between 0.10 and $0.42 \text{ g PO}_4\text{-P} \cdot \text{m}^{-3}$ and between 0.33 and $0.91 \text{ g tot-P} \cdot \text{m}^{-3}$. Tot-N for these plants varied between 11.6 and $25.1 \text{ g} \cdot \text{m}^{-3}$ and inorganic N between 8.3 and $19.6 \text{ g} \cdot \text{m}^{-3}$. Based on these values inorganic N/P ratios would be from 25 to 83 and total N/P ratios between 20 and 50. A large fraction of the phosphorus in tertiary effluent can be in particulate form. The availability of this fraction for algae is somewhat uncertain (Källqvist 1975).

Unfortunately, no analyses of the chemical composition of phytoplankton from the Öresund have been made. Samples from the Gulf of Finland and the Baltic gave a mean N/P ratio of 6.0 (Sen Gupta and Koroleff 1973). An often-cited ratio for oceanic conditions is 7.2 (Redfield 1958). For 25 samples of different composition collected at regular intervals during more than a year in the Trondheimsfjord, Haug et al. (1973) found a mean quotient of 7.3 with a standard deviation of 0.5. Thus it seems that Baltic, and probably also Öresund, phytoplankton has a N/P quotient somewhat lower than that of oceanic phytoplankton.

Chlorophyll α peaks in October/November 1976 and March/april 1977 were associated with a decrease in inorganic nitrogen and phosphate in proportions 10:1 (Fig 3 and 4). Inorganic nitrogen and phosphate were utilized in the bag experiments (from the start to chlorophyll α maxima) in quite variable proportions (Tab 6). The most extreme quo-

tients would probably not be maintained in longer-lasting experiments.

In mechanically-treated sewage/Baltic water mixtures phosphate was not consumed totally at the chlorophyll *a* maxima. If enriched water were to be added to the bag continuously, one would expect phosphate to accumulate in the medium and nitrogen to become limiting. Conversely, there were unused quantities of inorganic nitrogen in exp a for the chemically-treated sewage/Baltic water mixtures, indicating phosphorus limitation. In exp b both nitrogen and phosphorus were depleted by the time of chlorophyll *a* peaks for bags with secondary and tertiary sewage. Thus, the quotient between long-term uptake of the two elements would be between 5.7 and 13.3 (Tab 6). This seems reasonable considering the N/P ratio of phytoplankton and the consumption quotient around 10 found for chlorophyll *a* peaks in the Öresund.

Nutrient concentrations in the Öresund are mainly functions of conditions in the south Baltic and sewage discharge directly to the Öresund. Nutrient conditions at the south entrance to the Öresund give an indication of nutrient concentrations in the Öresund before enrichment with sewage, as the water in upper layers usually flows in a northerly direction. These background concentrations are generally lower than concentrations found in the central Öresund (Dahl-Madsen 1980, von Wachenfeldt 1980). Although mean annual concentrations of different nitrogen and phosphorus fractions in central Öresund were higher than corresponding concentrations in the south Baltic, quotients between N and P were not very different (Tab 7). Based on inorganic fractions, the N/P quotients were around 3, and for total N and P quotients were around 9.

Considering tot-N/tot-P ratios nitrogen would not appear to limit phytoplankton production in the Öresund and south

TAB 7. NUTRIENT CONCENTRATIONS ($\text{MG} \cdot \text{M}^{-3}$) AND N/P RATIOENTS (w/w) FOR SOUTH BALTIC (B) AND ÖRESUND (Ö) WATER 1974-77 ACCORDING TO MEAN VALUES IN WACHENFELDT (1980).

	1974		1975		1976		1977		MEAN 74-77	
	B	Ö	B	Ö	B	Ö	B	Ö	B	Ö
$\text{PO}_4\text{-P}$	14	37	24	24	17	22	14	21	17	26
TOT-P	40	47	35	39	29	41	26	39	32	41
INORG-N	46	89	44	83	50	64	42	81	45	79
TOT-N	280	350	285	440	265	350	290	340	280	370
$\text{INORG-N/PO}_4\text{-P}$	3.4	2.4	1.8	3.5	3.0	3.0	3.1	3.9	2.7	3.1
TOT-N/TOT-P	7.1	7.5	8.1	11.3	9.1	8.6	11.2	8.7	8.6	8.9

Baltic. Part of the nitrogen and phosphorus is probably not available to phytoplankton, however, e.g. nitrogen in "humic matter" (see Sen Gupta 1973). Calculations based on inorganic fractions are therefore more relevant. The mean inorganic N/P ratio in the Öresund is lower than both the N/P ratio in Baltic plankton and the probable uptake ratio. Thus phosphate should be left in the water when nitrogen has been consumed. Sen Gupta (1972) showed that when nitrate had disappeared in the upper 20 m (photic layer) of the south Baltic, phosphate was still present (mean about $5 \text{ mg P} \cdot \text{m}^{-3}$).

Optimal sewage treatment policy to reduce eutrophication in the Öresund

Mechanical and biological treatment would probably maintain nitrogen as the most limiting nutrient in the recipient. Assuming that tertiary treatment (phosphate precipitation could produce an effluent with an inorganic N/P quotient of e.g. 50, would phosphorus become limiting in the Öresund? Would it instead be better to maintain secondary treatment and introduce nitrogen removal, e.g. through denitrification (Ericsson 1975), ammonia stripping (Neretnieks et al

1973), or the marine aquaculture system of Goldman et al. 1974 a)?

More than 2 million people contribute to municipal sewage entering the Öresund directly or through tributaries. This population produces about $250 \times 10^6 \text{ m}^3$ wastewater annually (Damgaard 1980). Net outflow through the Öresund is estimated to about 120 km^3 annually, i.e. $120 \times 10^9 \text{ m}^3$ (Nielsen 1976). Total northmoving water transport, on the other hand, is on the order of $500 \times 10^9 \text{ m}^3$ annually (Dahl-Madsen 1980). The dilution factor for sewage would thus be between 500 and 2000.

Assuming all sewage to be untreated, or mechanically or biologically treated (the case around 1975), effluent concentrations are $20 \text{ g N} \cdot \text{m}^{-3}$ and $5 \text{ g P} \cdot \text{m}^{-3}$. The increase in N and P through sewage in central Öresund would amount to between 10 and $40 \text{ mg N} \cdot \text{m}^{-3}$ and 2.5 to $10 \text{ mg P} \cdot \text{m}^{-3}$ for the above dilution factors. The actual increase observed in central Öresund is on the order of $10 \text{ mg tot-P} \cdot \text{m}^{-3}$ and $50\text{--}100 \text{ mg tot-N} \cdot \text{m}^{-3}$ (Dahl-Madsen 1980).

The total amount of phosphorus and nitrogen released to the Öresund in 1977 was 5 800 and 25 000 tons, respectively (Damgaard op. cit.). If these amounts were evenly distributed into the gross outflow of water from the Öresund ($500 \times 10^9 \text{ m}^3$ per year), phosphorus concentrations would increase with 12 and nitrogen with $50 \text{ mg} \cdot \text{m}^{-3}$. These observed and calculated increases are also consistent with horizontal spreading calculations for point sources (Wändahl 1980). The calculations above are based on the assumption that the elements behave conservatively, i.e. residence time in the recipient is the same as that of water. In polluted lakes and eutrophicated estuaries this seems to be roughly true for phosphorus (Ahlgren 1980, Ryther and Dunstan 1971), but probably not for nitrogen due to denitrification and perhaps also nitrogen fixation.

For secondary sewage, sewage with phosphorus removal and sewage with nitrogen removal mixed in a recipient containing $40 \text{ mg inorganic-N}$ and $10 \text{ mg PO}_4\text{-P}\cdot\text{m}^{-3}$, it is possible to achieve phosphorus limitation only at low dilutions (Tab 8). It is assumed in these calculations that N, P and chlorophyll α are present in a fixed ratio in algae. Nitrogen removal to e.g. $5 \text{ g N}\cdot\text{m}^{-3}$, while phosphorus is maintained at the secondary treatment level, is advantageous at higher (and more realistic) dilutions. Thus nitrogen removal would depress algal production in central Öresund, while phosphorus removal would have no effect. This conclusion is strengthened by laboratory experiments showing that the N/P ratio in media for continuous culture of phytoplankton has a decisive effect on nutrient limitation. There are no additive or multiplicative effects between the two nutrients with respect to limitation (Rhee 1978).

TAB 8. NUTRIENT CONCENTRATIONS AND N:P QUOTIENTS AFTER ENRICHMENT AND THEORETICAL CHLOROPHYLL α YIELD ASSUMING COMPLETE TRANSFORMATION OF THE MOST LIMITING DISSOLVED NUTRIENT TO PHYTOPLANKTON BIOMASS. ÖRESUND WATER ($40 \text{ mg N}\cdot\text{m}^{-3}$, $10 \text{ mg P}\cdot\text{m}^{-3}$) WAS ENRICHED WITH THREE TYPES OF SEWAGE AT THREE LEVELS AS SHOWN. N:P:CHL α IN PHYTOPLANKTON WAS ASSUMED TO BE 12:2:1 BY WEIGHT. ALL NUTRIENT CONCENTRATIONS REPRESENT DISSOLVED INORGANIC FRACTIONS

SEWAGE TYPE	N P		100:1					DILUTIONS					500:1					2500:1				
			N	P	N/P	LIM. NUTR.	CHL α	N	P	N/P	LIM. NUTR.	CHL α	N	P	N/P	LIM. NUTR.	CHL α					
	G·M ⁻³	MG·M ⁻³				MG·M ⁻³	MG·M ⁻³				MG·M ⁻³	MG·M ⁻³				MG·M ⁻³	MG·M ⁻³					
MECH/BIOLOG	20	5	240	60	4.0	N	20	80	20	4.0	N	6.7	48	12	4.0	N	4.0					
P-REMOVAL	20	0.4	240	14	17.1	P	7.0	80	11	7.4	P	5.4	48	10	4.8	N	4.0					
N-REMOVAL	5	5	90	60	1.5	N	7.5	50	20	2.5	N	4.2	42	12	3.5	N	3.5					

To complete the picture one must look at what fractions of the discharge of N and P to the Öresund can be controlled. In 1975 there was practically no tertiary treatment in the region, and Swedish fertilizer industries discharged massive amounts of phosphate. The sum of all sources was around 6 000 tons of P annually. Of this over 90 % could probably be eliminated through sewage treatment and other measures, leaving 500 tons. The increase in phosphorus

would then amount to only $1 \text{ mg P} \cdot \text{m}^{-3}$ with "complete" dilution.

For nitrogen the picture is not so favourable. Of the more than 20 000 tons discharged into the Öresund, probably about half is from diffuse sources (precipitation, agricultural and urban run-off, lakes and forests) (Damgaard op. cit.). With an efficient (>80 %) nitrogen removal in sewage treatment plants, the total discharge could be reduced to about 14 000 tons per year, giving an increase of about $30 \text{ mg N} \cdot \text{m}^{-3}$. A large fraction of the diffuse nitrogen discharge is via agricultural drainage. Lower application of fertilizer is probably the only way to decrease this.

With very efficient P removal but no specific measures to reduce nitrogen, 1 mg P and $50 \text{ mg N} \cdot \text{m}^{-3}$ would be added to Öresund water. Using a background concentration of 40 mg N and $10 \text{ mg P} \cdot \text{m}^{-3}$, the N/P quotient after this addition would be about 8 which does not indicate phosphorus limitation. With nitrogen removal, but no specific phosphorus elimination, the quotient would be about 3.5 indicating nitrogen limitation. Thus potential chlorophyll *a* concentrations would be lower in the latter case.

One argument against nitrogen removal is that nitrogen can be fixed from the atmosphere by blue-green algae. Especially with an excess of phosphate and no inorganic nitrogen, blue-green algae can appear in massive amounts. Release of phosphorus stored in sediments can give this situation in lakes which have been freed from sewage (Leonardson and Bengtsson 1978, Ryding 1978, Forsberg 1978). Nitrogen-fixing blue-green algae are found especially in late summer in the Baltic, coinciding with low inorganic nitrogen concentrations. The algae are stimulated by phosphate additions, and nitrogen-fixation is inhibited by inorganic nitrogen (Granéli

and Sundström in prep.). The magnitude of nitrogen fixation under present phosphate conditions has not been measured in the Öresund, but it seems likely that fixation is of minor importance. In the Öresund *Nodularia spumigena*, *Aphanizomenon flos-aquae* and species of *Anabaena* are at the limit of their salinity tolerance. Mass occurrences of blue-green algae often seen in the Baltic south of the Falsterbo Channel are extremely scarce in the Öresund.

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